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Anaerobic digestion of Organic Matter

Biogas production from energy crop residues (Maize Stalks)

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Dissertação para obtenção do grau de Mestre em

Engenharia do Ambiente

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Resumo

Palavras-chave: energias renováveis, digestão anaeróbia, resíduos de milho, biogás, pré-tratamento.

Foram feitas experiências à escala laboratorial com caules de milho, aplicando alguns pré-tratamentos para elevar a produção de biogás na digestão anaeróbia, devido ao seu teor em metano e às suas propriedades energéticas.

As biomassas sofreram pré-tratamento químico (diferentes concentrações de amónia: 1%, 1,5%, 2% e 4%), mecânico (redução do tamanho das partículas) e/ou térmico (cozedura de pressão).

A experiência foi realizada em reactores batch e as amostras foram incubadas a 40ºC (±1ºC), durante 50 dias. A percentagem de metano no biogás observada foi entre os 51-72,5%.

O valor mais alto de biogás e metano foi encontrado na biomassa pré-tratada com 2% de amónia (467,570 L_N/kg_{SV} e 280,265 L_N/kg_{SV}, respectivamente) e o valor mais baixo de biogás e metano foi encontrado nos resíduos de milho não embalados e pré-tratados mecanicamente (180,81 L_N/kg_{SV} e 82,764 L_N/kg_{SV}, respectivamente).

Com base nos valores de biogás obtidos nesta experiência, em 2010 poderiam ser produzidos 100,33 GWh de energia, se toda a produção de milho produzida em Portugal fosse usada, o que corresponderia a 0,36% da energia renovável total produzida nesse ano.

Abstract

Keywords: renewable energy, anaerobic digestion, maize stalk, biogas, pretreatment.

The agricultural sector is one of the most interesting for effective dissemination of large-scale use of renewable energy, especially anaerobic digestion of feedstock from plant and animal origin. Laboratory-scale experiments were carried out with maize stalks, by applying some pretreatments to enhance its specific biogas yield in anaerobic digestion, due to its methane content. Feedstock were pretreated chemically (different ammonia concentrations), mechanically (particles size reduction) and/or thermally (pressure cooking). The methane percentage in biogas was between 51-72.5% and it was evident that methane yield had the same trend as biogas yield.

The highest value of biogas and methane were found on maize stalks just pretreated with 2% NH_3 (467.570 L_N/kg_{VS} and 280.265 L_N/kg_{VS}, respectively) and the lowest were found on maize stalks unwrapped pretreated mechanically (180.81 L_N/kg_{VS} and 82.764 L_N/kg_{VS}, respectively). The results show that all the pretreatments done were effective in enhancing methane yield, being the best feedstock the one pretreated with 2% NH_3 and the best pretreatment, the one where it was not preformed any other pretreatment. Based on the value of the biogas yield obtained in this experiment, 100.33 GWh of energy can be produced from energetically available amount of maize stalk in Portugal, using 2010 data.

Resumo

Palavras-chave: energias renováveis, digestão anaeróbia, resíduos de milho, biogás, pretratamento.

Existe muita investigação a ser desenvolvida, no âmbito das energias renováveis, tal como a desenvolvida por nós, em que usámos resíduos de milho (caules) para produzir biogás, através de digestão anaeróbia.

Foram feitas experiências à escala laboratorial com caules de milho, aplicando alguns pré-tratamentos para elevar a produção de biogás na digestão anaeróbia, devido ao seu teor em metano e às suas propriedades energéticas.

Os caules de milho são materiais lenho-celulósicos difíceis de degradar, como tal é necessário efectuar algum(s) pré-tratamentos para facilitar a sua digestão.

As biomassas sofreram pré-tratamento químico (diferentes concentrações de amónia: 1%, 1,5%, 2% e 4%), mecânico (redução do tamanho das partículas) e/ou térmico (cuzedura de pressão).

A experiência foi realizada em reactores batch anaeróbicos sem alimentação contínua, cheios com uma parte de inoculo, água desionizada e biomassa pré-tratada. As amostras foram incubadas a 40 ºC (±1 ºC) em duas câmaras com temperatura controlada, durante 50 dias. Os sacos impermeáveis a gases (alluminium) ligados a cada batch, eram lidos quando cheios e davam os valores de biogás e metano produzido pela biomassa a testar. A percentagem de metano no biogás observada foi entre os 51-72,5% a produção de metano apresentava a mesma tendência que a produção de biogás.

Analisando os dados obtidos, o valor mais alto de biogás e metano foi encontrado na biomassa pré-tratada com 2% de amónia (467,570 L_N/kg_{VS} e 280,265 L_N/kg_{VS}, respectivamente) e o valor mais baixo de biogás e metano foi encontrado nos resíduos de milho não embalados e pré-tratados mecânicamente (180,81 L_N/kg_{VS} e 82,764 L_N/kg_{VS}, respectivamente).

Os resultados obtidos mostraram o tipo de biomassa influencia a produção de biogás e que todos os pré-tratamentos efectuados foram eficazes em aumentar a produção de metano, sendo a melhor biomassa, aquela que foi tratada com 2% de amónia e o melhor tratamento, aquele em que não se efectuou qualquer outro tipo de pré-tratamento.

Com base nos valores de biogás obtidos nesta experiência, em 2010 poderiam ser produzidos 100,33 GWh de energia, se toda a produção de milho produzida em Portugal fosse usada, o que corresponderia a 0,36% da energia renovável total produzida nesse ano em Portugal.

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Abbreviations

AD – Anaerobic digestion.
ADF – Acid detergent fibers.
ADL – Acid detergent lignin.
APA – Portuguese environmental agency.
BOD – Biological Oxygen Demand.
CELL – Cellulose.
CHP – Combined Heat and Power.
C – Carbon.
CH4 – Methane.
CO_2 – Carbon dioxide.
EDP – Energias de Portugal.
EU – European Union.
GHG – Greenhouse Gases.
GWP – Global warming potential.
H_2 – Hydrogen.
HEMI – Hemicellulose.
INE – National statistics institute..
LIGN – Lignin.
MS1 – Maize stalks pretreated wit 1% NH3.
MS1.5 – Maize stalks pretreated with 1.5% NH3.
MS2 – Maize stalks pretreated with 2% NH3.
MS4 – Maize stalks pretreated with 4% NH3.
MSU – Maize stalks unwrapped.
MSW – Maize stalks wrapped.
NaOH – Sodium hydroxide.
NDF – Neutral detergent fibers.
N_2O – Nitro Oxide.
NH_3 – Ammonia.
NH_4 – Ammonium ion.
OM – Organic matter.
RES – Renewable Energy Source.
TN – Total nitrogen.
TS – Total solids.
UN – United Nations.
UR – Urban residues.
VS – Volatile solids.
WECD – World Commission on Environment and Development.

1. Introduction

1.1 Motivation

Nowadays, there is a real concern about the environment, due to its importance to a sustainable future. The demand for a better way of living, as well as mitigation of environmental, leads us to the search of new kinds of technologies, habits and ways of thinking. As Richard Wright says: ”In the light of global climate change, moving in the direction of renewable energy sources seems essential”.

Almost every country is searching for new ways to produce and new sources of “green energy”, as an attempt to face the increasing oil prices and to reduce the pollution problems caused by those industries, as well as by reducing the consumption of a resource which is limited. In 2010, Portugal produced 28 TWh of energy from renewable sources (mostly from hydric, wind and solar), this represents almost 52% of the energy produced in Portugal in this year [6], which is not still enough, since Portugal produce only 15% of what it is consumed.

The agricultural sector has long been recognized as one of the most interesting activities for effective implementation of a large-scale use of renewable energy, especially feedstock conversion from plant and animal origin.

There is an opportunity for the agriculture in the field of energy production. This arises from the environmental issues direct or indirectly connected with agriculture practice, from Portugal energy dependence from third countries and from the economical crisis of the agricultural sector related to food markets.

One of the technologies which can be used is biogas production via anaerobic digestion (AD) from organic residues. AD can operate using manure (from livestock activities) and energy crops and/or other crops residues, like maize stalks, to produce energy. One of the residues being studied are the residues from crops because this feedstock input used in AD derives from crops used for animal food, and their residues still have enough energy stored and capable to be transformed by AD into biogas (Schievano et al., 2009). This will help to minimize the problems caused by agricultural sector.

The biomass used to fuel the AD, must have some specific composition that demand some measuring before being used. Since most of them are lignocellulosic plants, pretreatment(s) are needed to enhance it energetic content, in order to maximize biogas production.

In this work, the main objective was to verify, in a laboratory scale, if the application of some pretreatments on lignocellulosic crop residues (maize stalks) increases the biogas and methane production from this kind of feedstock, with the intent to transform it into energy.

1.2 Residues and energy issue

A residue is defined as any kind of material (solid, liquid or gaseous), to which it is not attributed any significant value to justify it conservation. Residue production is a big environmental problem, due to the volume produced, variability and complexity of it characteristics/composition.

The organic residues problem has a huge role in the modern society, because production increases every year. Moreover laws for residues management are becoming increasingly severe and the place predestinated to storage/disposal, is getting shorter every day.

Environmental problems are evident in particular from agricultural sector, where farming has increased up to industrial dimensions, sometimes with great imbalances between crop and livestock production.

One of the most important issues is the production of manure in quantities well above the disposal capacity of the soil. The disposal of animal manure leads to a problem of soil pollution. If excessive amounts of manure are widespread on a few hectares of agricultural soil, lead to a more severe alteration of environmental equilibrium.

So, to minimize this problem, a good waste management is needed, and this involves a hierarchical management, where on the top is the most important step, prevention, with the responsibility of the residue producers/owners and in the innovation of products using ecodesign, leading to a lower residue production. Other measure is the materials reuse, by recycling and valorization processes. Finally and only when the other actions are not possible, the residue must be eliminated, under proper control.

According to APA (Portuguese environmental agency), “The waste management policy is based on objectives and strategies to ensure the preservation of natural resources and minimize negative impacts on public health and on the environment … should be encouraged to reduce waste production, reuse and recycling by rows. To a large extent this is to promote the identification, design and adoption of cleaner technologies and products and recyclable materials. In addition to prevention, it remains to promote and develop integrated systems of collection, treatment, recovery and disposal of waste per row.”

The first impression is that elimination may be the quickest way to solve the problem, but in reality, it is still a problem to the environment, because those residues have great quantities of water and other compounds which decompose. Humidity percentage leads to soil pollution, methane production, contamination of the aquifers, and many other problems.

To solve these problems, the residues can be treated, through the application of a treatment technique or a combination of techniques, depending on the residue composition.

Another problem faced by mankind is the search for new energy sources. Nowadays,

the main primary energy source is coal or petroleum (fossil or non-renewable source), and the demand for renewable energy sources is a need to end the non-renewable sources dependency and all the problems that come with it, like greenhouse gases concentration increase.

Many renewable energy forms appeared recently, to decrease the demand in fossil energy and reduce the greenhouse effect. One of them is bioenergy. Bioenergy technologies, normally uses the feedstock as a fuel, to produce energy (thermal or electric). In this case the fuel is a biofuel, and since the input materials are plants, the uptake of CO_2 reduces the greenhouse effect.

However, bioenergy introduced another problem, because most of the bioenergy produced, uses energy crops as a feedstock input (ex. maize, sugarcane, wheat), that could be used as food, instead of a material input to produce energy. This situation, in the beginning, raised the food prices, turning it into a social-economic problem. One way to solve this and other problems, like storage/energy/fuel problems, is using the crop residues as input materials, because they still have OM enough to produce biogas, still their feeding role is guaranteed.

1.3 Carbon cycle and uptake

Carbon (C) is one of the four most abundant elements on Universe. Almost everything is made with C. There are two forms of C, the organic (living and dead organisms) and the inorganic form (rocks).

On Earth, C moves through the oceans, atmosphere and geosphere, in a big biogeochemical cycle which divided in two types, slow cycle (geological) and the fast cycle (biological). The slow cycle is represented in a million-year scale and is integrated in the planet structure, with its beginning at the same time as the solar system formation. Almost all of the C is found in lithosphere, mostly inorganic C, because the organic C is stored on the fossil fuels deposits. The cycle begins with the CO_2 stored in the atmosphere, reacting with the water, forming carbonic acid, which reacts with the calcium and magnesium from the calcareous rocks. The erosion effects due to precipitation take the carbonates formed into the sea. These carbonates, in the end, deposit on the bottom by layers and form the sedimentary rocks. When the tectonic plates are renewed by subduction processes, the materials (sedimentary rocks formed) react between them and release CO_2. The cycle is completed, when the CO_2 is released to the atmosphere by volcanism phenomena.

The fast cycle is renewed every 20 years. Without anthropogenic interference, the cycle is composed by 3 stocks: terrestrial (20 000 Gt), atmosphere (750 Gt) and oceans (40 000 Gt). This cycle plays an important role in the carbon flows between the pools, through the respiration and photosynthesis processes.

In the photosynthesis process, plants and algae take solar energy and CO_2 from the atmosphere and produce oxygen and carbohydrates, which are the bases for plant growth and development. Animals, plants, bacteria and fungi, use C in the respiration and organic decomposing process, releasing CO_2 to the atmosphere. The equations to these processes are:

Photosynthesis:

$$6CO_2 + 6H_2O + \text{energy (sun light)} \rightarrow C_6H_{12}O_6 + 6O_2$$

Respiration:

$$C_6H_{12}O_6 \text{ (organic matter)} + 6O_2 \rightarrow 6CO_2 + 6\ H_2O + \text{energy}$$

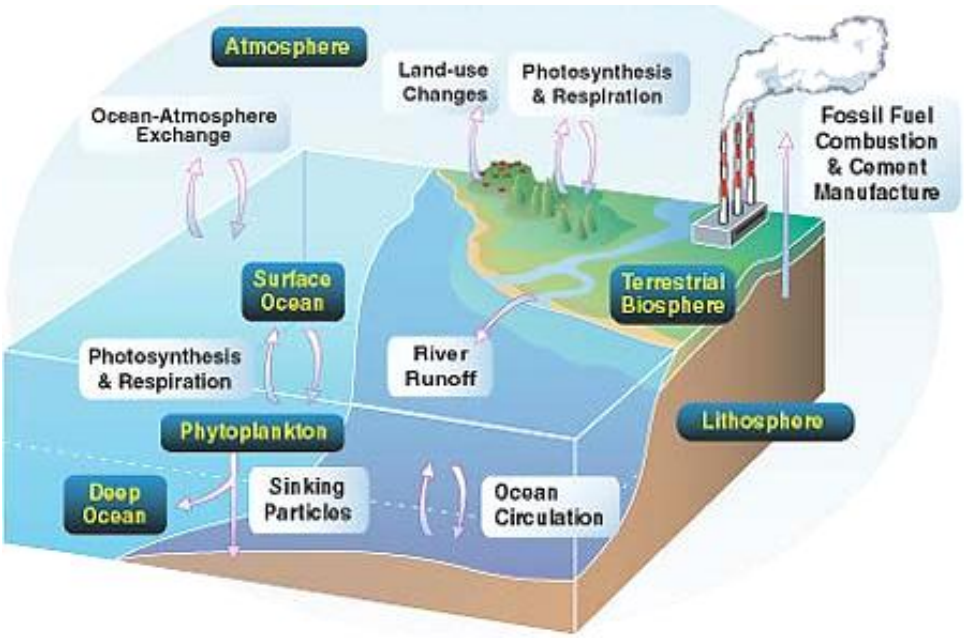
Even if the atmospheric pool is the smallest, this pool is the one which determines the CO_2 concentration in the atmosphere, and could be responsible for the climatic changes.

Other natural process that adds CO_2 to the atmosphere are natural fires, by burning the feedstock and OM and by destroying plants that will also decompose and form CO_2.

The anthropogenic influence increases the CO_2 concentration in the atmosphere, increasing, also the atmospheric temperature. The quantities of CO_2 emitted to the atmosphere are higher than the quantities that can be naturally removed by the cycle – by C sedimentation. This is the result of burning fossil fuels and deforestation (that were two of the ways to remove C), caused by Man.

The oceans represent the largest pool, which is 50 times greater than the atmospheric. There are transfers between these two pools (picture 1), through chemical processes that establish a balance between the surface layers of the oceans and the concentrations in the air above the surface. The amount of CO_2 absorbed by the oceans depends on the temperature and the concentration already present. Low temperatures of the ocean surface potentiate a greater absorption of atmospheric CO_2, while warmer temperatures can cause the emission of CO_2.

The differences on the fast cycle are explained by the respiration and photosynthesis processes: the ocean life consumes huge quantities of CO_2, and the respiration and photosynthesis are fast processes. Since the phytoplankton is consumed by the zooplankton in a few days, small quantities of C are deposited in the bottom of the ocean, and in a long time, it represents a sink of CO_2 collected from the atmosphere. Another sink of CO_2 from the atmosphere is the transformation of OM into fossil fuels, when the photosynthesis is higher than the respiration and the OM slowly forms sedimentary deposits, and is decomposed in an anaerobic process for millions of years.



Picture 1 - Carbon cycle (Barkeley lab, 2010).

1.4 Greenhouse gases and global warming

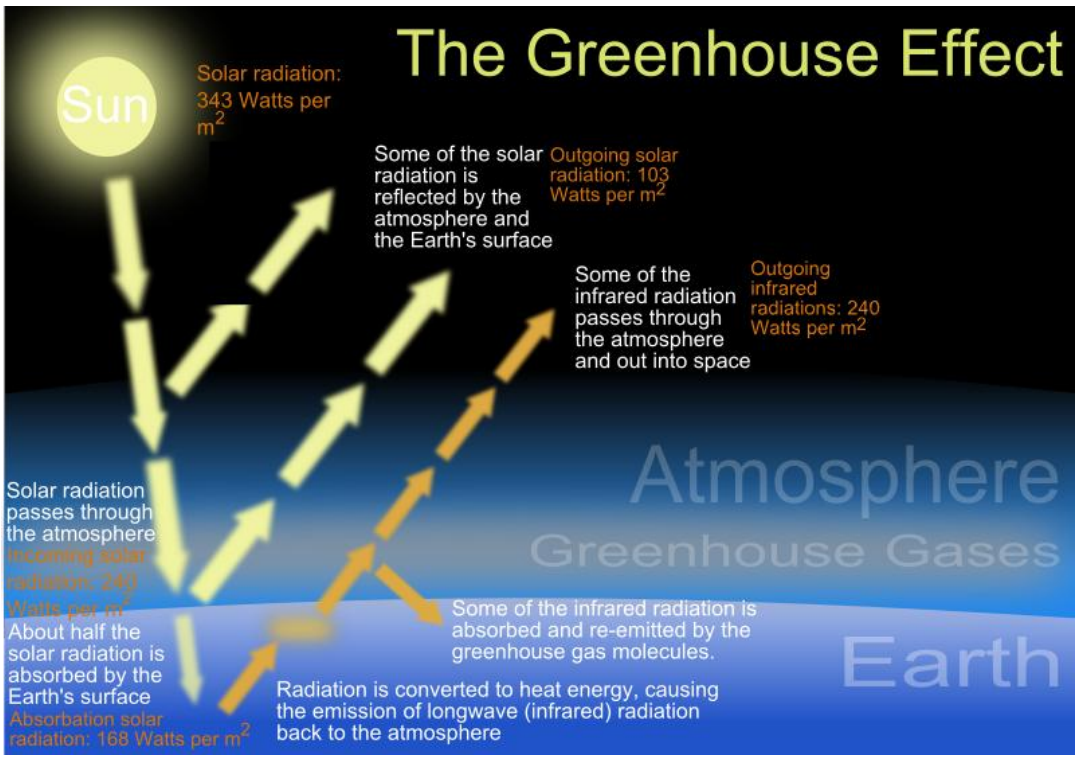
Since the beginning of the industrial revolution, the burning of fossil fuels and deforestation has contributed to the increase CO_2 in the atmosphere, despite the uptake of a large portion of the emissions through various natural "sinks" involved in the carbon cycle mentioned above (picture 1).

Greenhouse gases (GHG) are present in atmosphere, absorbing and emitting radiation within the thermal infrared range, being the main cause of the greenhouse effect. The most abundant GHG on Earth's atmosphere are: water vapor (36-70%), CO_2 (9-26%), methane (4-9%), nitrous oxide (N_2O) and ozone (3-7%). GHG, such as N_2O, CO_2 and methane (CH_4), affect the global environment and climate while ammonia (NH_3) contributes to general atmospheric pollution (Olesen, 2005).

Global warming is the continuing rise in the average temperature of Earth's atmosphere and oceans and is caused by increased concentrations of GHG in the atmosphere, resulting from human activities such as deforestation and burning of fossil fuels. An increase in global temperature will cause sea levels to rise and will change the amount and pattern of precipitation, and a probable expansion of subtropical deserts. Warming is expected to be strongest in the Arctic and would be associated with continuing retreat of glaciers, permafrost and sea ice.

Atmospheric concentrations of greenhouse gases are determined by the balance between sources (emissions of the gas from human activities and natural systems) and sinks (the removal of the gas from the atmosphere by conversion to a different chemical compound) (IPCC, 2007).

The major non-gas contributors to the Earth’s greenhouse effect are the clouds which absorb and emit infrared radiation and thus have an effect on radiative properties of the atmosphere.



Picture 2 – The greenhouse effect (Zoofari, 2009).

The greenhouse effect in picture 2 is a process by which thermal radiation from a planetary surface is absorbed by atmospheric GHG, and is re-radiated in all directions. Since part of this re-radiation is back towards the surface, energy is transferred to the surface and to the lower atmosphere. As a result, the temperature becomes higher than it would be if direct heating by solar radiation were the only warming mechanism.

Solar radiation at the high frequencies of visible light passes through the atmosphere to warm the planetary surface, which then emits this energy at the lower frequencies of infrared thermal radiation. Infrared radiation is absorbed by GHG, which in turn re-radiate much of the energy to the surface and lower atmosphere, thus warming the Earth surface.

Newly developed and currently available technologies including renewable energy (such as solar power, tidal and ocean energy, geothermal power, and wind power) and more controversially nuclear power and the use of carbon sinks, carbon credits, and taxation are aimed more precisely at countering continued GHG emissions.

AD is a good candidate technology to low GHG emissions and global warm potential (GWP), since the biogas (CO_2, CH_4 and other gases) becomes from organic sources with a short carbon cycle. AD technologies reduced GHG emissions and global warming potential (GWP) by approximately 79% (Turnbull, J. H. and Kamthunzi, W., 2004).

2. Biogas as bioenergy

The next four chapters (2.1 – 2.4) describe how biogas is produced, the kinds of feedstock that can be used to produce it, the types of pre-treatments possible to apply, description of AD and a characterization of biogas, where the maize crop residues are in focus, due to the laboratorial component of this thesis.

This process (AD) is used by Man with an objective, produce biogas by degrading organic matter from residues and/or feedstock with the help of microorganisms. For that, feedstock can be pretreated to maximize the biogas production which will increase process yield.

The biogas production is followed by a series of steps until the final product formation (picture 3).

Picture 3 – Biogas production stages.

2.1 Biomass

Biomass is carbon, hydrogen and oxygen based material. Biomass for renewable energy-production is defined as a biological material derived from living, or recently living organisms, which can be used as biofuel [2]. Biomass can be obtained as products and residues from aquatic plants, agriculture, forest residues and related industries and biodegradable fraction of industrial and urban residues.

To produce energy, biomass can be used directly or indirectly. It can be applied in direct burn, or used indirectly, as solid biofuel, for example raw material (harvested wood and wood waste streams, grass cuttings, domestic refuse, agricultural waste, non-food energy crops and dried manure), generating some heat or steam which can be converted into electricity or be used as domestic or industrial heat. If the raw materials are not in a suitable form to be burnt, they can be shredded and densified in a suitable form, like pellets (1-3 cm) (picture 4d) [4].

Considering biomass to be used indirectly there is a wide number of available technologies to transform the different biomass types into a renewable energy source. Thermo-chemical conversion technologies (pyrolysis or combustion) are the ones in which

heat is the dominant mechanism to convert the biomass into another chemical form. The basic alternatives of pyrolysis and gasification are separated mainly by the extent to which the chemical reactions involved are allowed to proceed (mainly controlled by the availability of oxygen and conversion temperature).

On the other hand, biomass can be converted to other usable forms of energy like biogas by using microorganisms mediated biochemical process to break down biomass.

Physical-chemical process like transesterification, converts vegetable oils into biodiesel, can be produced from left-over food products like vegetable oils and animal fats. Ethanol is derived primarily from the fermentation of energy crops like sugarcane and maize. Both can be used directly as a fuel or as an additive to a fossil fuel like diesel/gasoline, which can be burned in a CHP to recover electricity and heat.

In many of these processes, energetic crops can be used directly as an input material to produce energy or the residues produced as a byproduct, instead. An energy crop is a specific plant grown (picture 4a) to make biofuel, or to be directly burnt.

Energy crops are cultivated using conventional agriculture practices and can be used by the farmers along with other food production crops. The advantage of energy crops is not requiring the best land and need less water and fertilizers, since the quantity is more important than the production quality.

When biomass is produced in a renewable basis, using the energy produced by it does not imply that there is an increase in the net CO_2 levels, because the CO_2 released in the process is the one captured by the plant in it growth, it's a cyclic process.

2.1.1 Maize stalks as biomass input

Maize (*Zea mays* L.) is a lignocellulosic crop and it stalks are being used increasingly for heating, where specialized corn stoves are available and use either maize-meal or wood pellets to generate heat, due to it high levels of carbohydrates. Maize cobs are also used as a biomass fuel source. Maize is relatively cheap and home-heating furnaces have been developed which use maize kernels (picture 4b) as a fuel.

Maize is also increasingly used for the production of ethanol fuel. Ethanol is mixed with gasoline to decrease the amount of pollutants emitted when used to fuel motor vehicles, because less gasoline is combusted. High fuel prices in mid 2007 led to higher demand for ethanol, which in turn lead to higher prices paid to farmers for maize. This led to the 2007 harvest being one of the most profitable maize crops in modern history for farmers. Because of the relationship between fuel and maize, prices paid for the crop now tend to track the price of oil.

With the increasing use of maize in energy sector, a big problem arises. Food/feed problem is well-known, because a great part of the maize is being used to produce fuel or heat, instead of being used as human/animal food. After grain separation, maize stalks and straw are often burned in fields or feed to animals, without any energy recovery, while they could make a substantial contribution to the power supply if it is digested in a biogas plant.

Maize silage is widely used in Germany as a feedstock for biogas plants (Deublein, D. et al., 2008). Here the maize is harvested, shredded in small pieces then placed in silage clamps (picture 4c) from which it is fed into the biogas plants, where AD takes place, meanwhile it can be kept in bales (pictures 4e and 4f). This process makes use of the whole plant rather than simply using the kernels as in the production of fuel ethanol. The energy-efficient digestion, particularly of maize stalks or rice straw, seems to be a promising practice in developing countries. It would be economically interesting and would contribute to environmental sustainability.

Picture 4 – a) Maize field (Paranoid, 2004); **b)** Maize kernels (http://www.ecofriend.com); c) Maize harvest and shred process (Marilyn Peddle, 2007); **d)** Pellets (http://www.hearth.com); **e)** Maize Silage Bale (http://www.star-gro-products.ca); **f)** Wrapped bales (http://cliffordddennis agriculturalsupplies.co.uk).

2.1.2 Advantages and disadvantages

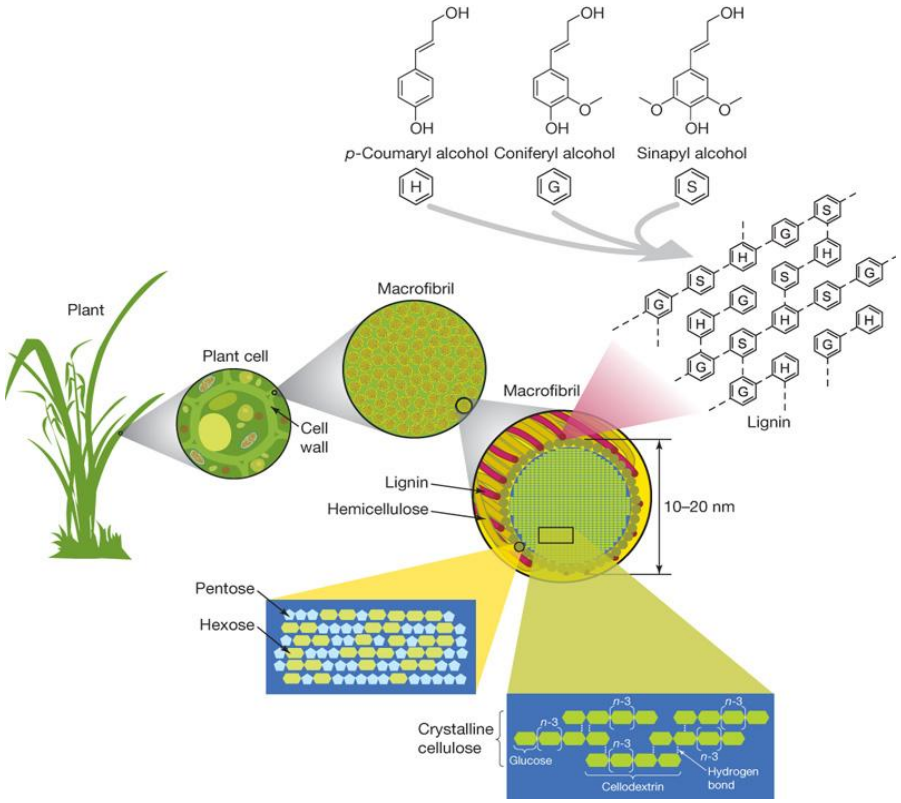
Advantages – Biomass for alternative energy use is very abundant. It can be found on every square meter of the earth as seaweed, trees or manure; It is cheaper than other energy sources and its production can often mean the restoration of abandoned soils; It may also promote the use of uncropped areas and provide jobs in rural communities; The growth of crops for biomass releases oxygen and uses CO_2 present in the air.

Disadvantages – It can take more energy to plant and harvest the crops than it is worth to get a net energy gain. It also takes up more water from the earth and other fossil fuels to make the fertilizers and fuels for planting and harvesting; Biomass crops are not available all year they are seasonal crops; Most of the biomass is used as input material to energy production, instead of as food.

2.2 Pre-treatments

To use biomass as a substrate to produce biogas and before putting the biomass in the digester, there are some parameters that need to be well-known to characterize the biomass in question, like hemicellulose (HEMI), cellulose (CELL), lignin (LIGN), volatile solids (VS), total solids (TS), ashes (ASH) and also protein and lipids content.

Most of the biomass from agricultural sector used as input in AD, is lignocellulosic (Picture 10). Lignocellulosic biomass materials, like maize stalks, are mainly composed by cellulose, hemicellulose, lignin, extractives, and several inorganic materials.



Picture 5 – Structure of lignocellulose (Rubin, E. M., Nature 454, 841-845 2008).

The cellulose chains are packed by hydrogen bonds in so-called 'elementary and microfibrils'. These fibrils are attached to each other by hemicelluloses, amorphous polymers of different sugars as well as other polymers such as pectin, and covered by lignin. The microfibrils are often associated in the form of bundles or macrofibrils (picture 5). This special and complicated structure makes cellulose solubilization resistant to both biological and chemical treatments.

Lignin is the most recalcitrant component of the plant cell wall, and the higher the proportion of lignin, the higher the resistance to chemical and enzymatic degradation

(Taherzadeh, M. J. et al., 2008).

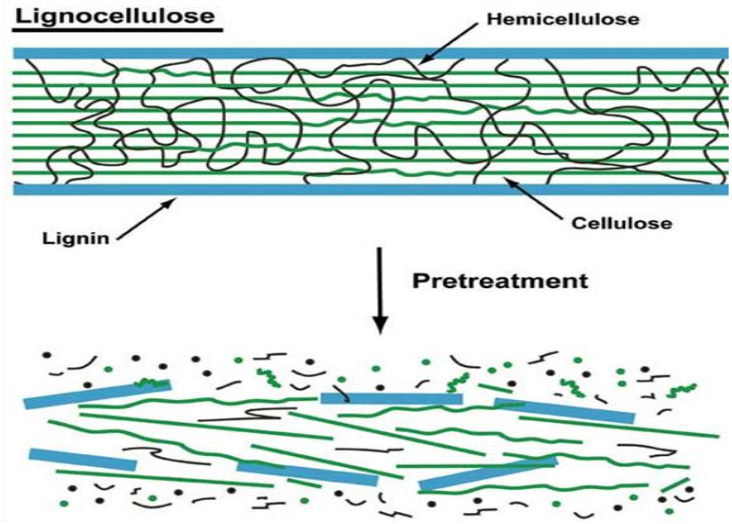
This composition makes lignocellulosic biomass more difficult to biodegrade and as consequence the AD less productive, resulting in a lower biogas yield. To improve biogas production, one or more pretreatments can be applied before inserting biomass in the digester, aimed at increasing the degradation of those molecules, breaking the strong bonds (picture 6), thus, fostering and accelerating the hydrolysis step.

A study for improvement of biogas production from rice straw showed that a combination of grinding, heating, and ammonia treatment (2%) resulted in the highest biogas yield (Zhang, R., 1999).

Most of the research in pretreatment of lignocellulosic biomass has been for ethanol production. One method of producing ethanol from lignocellulose involves reducing the size of biomass to smaller particles, and using acid or enzyme treatments to hydrolyze the biomass to sugars.

The same pretreatments can also be applied to biogas production, because the aim is hydrolysis, a step common in ethanol and biogas production. Several methods have been introduced for pretreatment of lignocellulosic materials prior to digestion. Pretreatment methods have a wide range of complexity, and can be combined together to further optimize AD performance (Shephard, T. A., 2006). These methods are classified into “Physical pretreatment”, “Chemical pretreatment”, “Thermal pretreatment” and “Biological pretreatment”.

The following sections describe the basic application of each method and the mechanisms providing the benefits to AD systems.

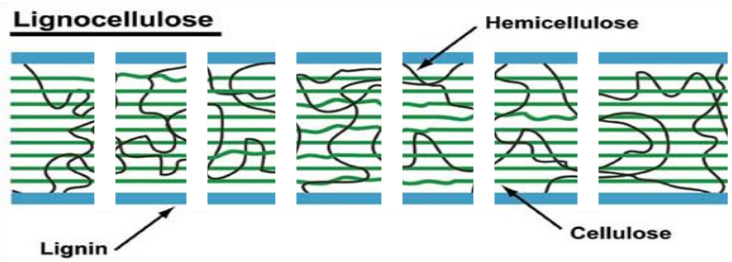


Picture 6 – Lignocellulose breakdown.

2.2.1 Physical pretreatments

Physical and mechanical pretreatments increase the accessible surface area and size of pores, and decrease the crystallinity and polymerization degrees of cellulose by reducing the biomass size (Angelidaki, I. et al., 2000). The size-reduction step is necessary to eliminate mass and heat-transfer limitations during the hydrolysis reactions.

Different types of physical processes such as chop, milling and irradiation can be used to improve the biodegradability of lignocellulosic residues materials. Chop pretreatment with a cutting equipment consists of simply reducing the biomass particles size into small pieces. This method allows to improve susceptibility to enzymatic hydrolysis, because reducing the size of the materials implies that the degree of crystallinity of lignocelluloses will decrease and the accessible surface area will increase (picture 7), facilitating the enzymatic activity, which improves it degradation towards an increase in biogas production.



Picture 7 – Schematic of physical pretreatment effect on lignocellulose.

Milling process uses a milling machine to reduce the biomass into smaller particles like powder (± 60-700 µm), more efficient than the chop method.

Irradiation by gamma rays, electron beam or microwaves can also improve biogas yield from lignocellulosic feedstock. The cellulose component of the lignocellulose materials can be degraded by irradiation to fragile fibers and low molecular weight oligosaccharides and even cellobiose. It could be due to preferential dissociation of the glucoside bonds of the cellulose molecular chains by irradiation in the presence of lignin. A very high irradiation can lead to the decomposition of oligosaccharides and the glucose ring structure.

2.2.2 Chemical pretreatments

Chemical pretreatments applied to lignocellulosic materials were originally developed and have been extensively used in paper industry for delignification, in order to produce high quality paper products. (Fan, L. T. et al., 1982).

Chemical pretreatments have the primary goal of improving the biodegradability of cellulose by removing lignin and/or hemicellulose, and to a lesser extent decreasing the degree of polymerization and crystallinity of the cellulose component. Chemical pretreatments are the most studied technique among pretreatment categories.

Alkaline pretreatment consists of the application of alkaline solutions such as sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) (lime) or ammonia (NH_3) and mixing it at a fixed temperature for a certain period, to remove lignin and a part of the hemicellulose, and efficiently increase the accessibility of enzyme to the cellulose.

The action mechanism is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, as lignin and other hemicellulose. It cause swelling of biomass, which subsequently leads to a decrease in the degree of polymerization and crystallinity, increased internal surface area, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates.

Among the bases investigated, ammonia has the highest potential for use in commercial processes since it can be recovered and recycled due to its high volatility. Thus it reduces chemical cost and residues treatment. (Fan, L. T. et al., 1987).

In comparison to other pretreatment technologies, alkali pretreatment usually uses lower temperatures and pressures, even ambient conditions. Pretreatment time, however, is recorded in terms of hours or days which are much longer than other pretreatment processes (Zheng, Y. et al., 2009).

NaOH has been shown to disrupt the lignin structure of the biomass, increasing the accessibility of enzymes to cellulose and hemicellulose. Sun, R. et al. (1995) found that for wheat straw, the optimal condition was to use 1.5% NaOH for 144 hours at 20°C, which resulted in 60% release of lignin and 80% release of hemicellulose.

The advantage of lime pretreatment is that the cost of lime required to pretreat a given quantity of biomass is lowest among alkaline treatments.

2.2.3 Thermal pretreatments

Thermal pretreatments subject biomass to high temperatures (>60 ºC) for a period of time that typically ranges from several minutes to several hours in a humid environment, in order to break the lignocellulosic bonds (Mladenovska et al., 2006).

Pressure cooking is a heat treatment in hot water, at high pressure, preformed in a special autoclave. Biomass is brought to high temperature (>100 ºC), leading to the cell wall disintegration, releasing the intracellular materials, obtain the effective delignification without sugar degradation. This technique may present some risks of environment acidification (pH 4), and produce inhibitory compounds capable to inhibit anaerobic digestion.

Thermal treatment may prove to be an economically sustainable option if the heat resulting from the biogas combustion can be utilized for the pre-heating process and AD heating.

2.2.4 Biological pretreatments

Microorganisms can also be used to pretreat the lignocelluloses and enhance enzymatic hydrolysis. The applied microorganisms usually degrade lignin and hemicellulose but very small part of cellulose, since cellulose is more resistance than the other parts of lignocelluloses to the biological attack. Several fungi have been used for this purpose. White-rot fungi are amongst the most effective microorganisms for biological pretreatment of lignocelluloses.

The biological pretreatment is a promising technique and has very evident advantages, including no chemical requirement, low energy input, mild environmental conditions and environmentally friendly working manner. However, as disadvantages, it is a very slow procedure, requires careful control of growth conditions and large amount of space to perform treatment, most of lignocellulosic degrading microorganisms solubilize/consume not only lignin but also hemicellulose and cellulose (Sun, Y. and Cheng J. J., 2002,).

2.3 Anaerobic Digestion

The anaerobic fermentation is a process which occurs spontaneously in nature, where organic substances are decomposed in the absence of oxygen. Man has simply developed the process in a controlled environment.

This phenomenon is a complex series of biological processes that uses microorganisms (table 1) to degrade organic matter in absence of oxygen (picture 8), stabilizing, reducing odor, pathogenic and mass, can be used to produce a type of biofuel called biogas, which

is formed as a byproduct. AD is capable of conserving nutrients with sludge stabilization while simultaneously producing renewable energy in the form of CH_4 which can be used for heating or electrical generation (Shepherd, T.A., 2006) or as fuel for transportation.

AD is composed by hydrolysis, acidogenesis, acetogenesis and methanogenesis processes. All of those steps transform organic matter's molecules, in even smaller pieces, producing in the end the biogas and digestate, and the products formed in the end of each step, is used in the following step. Digestate is one of the final by-products of organic matter anaerobic digestion (Tani et al., 2006; Zhang et al., 2007). The digestate is the undigested portion of the organic matter which bacteria cannot use and the remains of dead bacteria. It contains valuable plant nutrients like nitrogen, phosphorus, potassium and organic compounds, so it can be spread on the agricultural soils as a substitute for commercial fertilizer.

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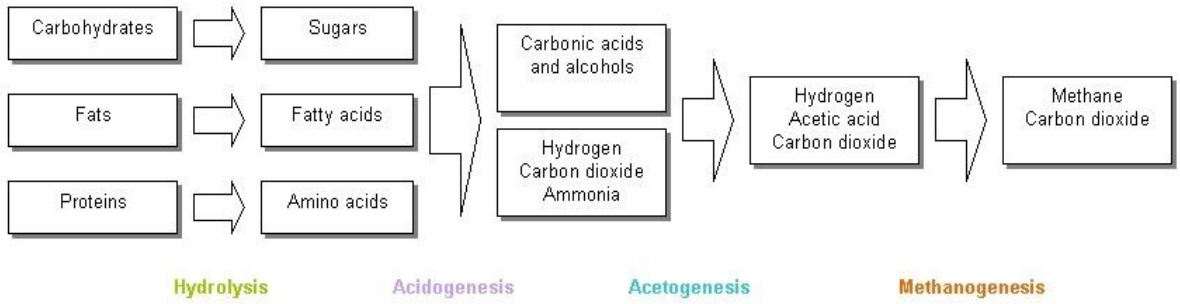


Picture 8 – Stages of anaerobic digestion (Alex Marshall, Clarke Energy).

In hydrolysis, hydrolytic bacteria produce and release extracellular enzymes to the environment promoting the breakdown of insoluble polymers like polysaccharides in smaller molecules, like organic acids, alcohols, H_2 and CO_2. Those bacteria also transform, by fermentation, proteins and lipids into similar compounds.

In acidogenesis, complex molecules (carbohydrates, lipids and proteins) depolymerized into soluble compounds by hydrolytic enzymes (cellulases, hemicellulases, amylases, lipases and proteases) are fermented into volatile fatty acids (acetate, propionate, butyrate and lactate), neutral compounds (ethanol and methanol), ammonia, H_2 and CO_2.

In acetogenesis, acetogenic bacteria transform the resulting products from the previous process, into acetic acid, ammonia, H_2 and CO_2.

The final stage is methanogenesis, in this biological process, the products formed in the previews stages are converted by methanogenic bacteria and used to produce biogas (mainly methane, $CO_2$and water), while hydrogen is consumed. This phase is sensitive to pH variation, being the optimal range between 7.2-7.5.

Table 1 - Some bacteria species involved in AD process.

Step	Specie/Gender
Hydrolysis	*Butyvibrio, Clostridium, Ruminococcus, Anaeroivibrio, Batteroidi, Bacillus*, *Homoacetogenic*, etc.
Acidogenesis	*Clostridium, Ruminococcus, Selonomonas, Desulphomonas, Syntrophomonas, Syntrophobacter*, etc.
Acetogenesis	*Eubacterium, Acetogenium, Clostridium*, etc.
Methanogenesis	*Methanobacterium*, Methanobrevibacter*,Methanococcus*, Methanomicrobium*, Methanogenium*, Methanospirillium hungatei,* etc

* - A lot of species.

2.3.1 Parameters that affect AD

A fundamental objective of any process control strategy is to maintain optimal operating conditions and stability. For maximum performance environmental conditions must be favorable to the metabolism of the bacterial strains involved in the process. In the case of anaerobic digestion, this concept becomes particularly significant since the phase controlling the entire process (methanogenesis) is particularly sensitive to environmental changes (Deublein, D. et al., 2008).

It should however be noted that the analysis of these parameters must be global as the variation of a single factor, if not accompanied by an overall monitoring of all the other parameters, is difficult to interpret.

Some of the parameters that affect anaerobic microorganisms are: oxygen concentration, pH, temperature, substrate composition and Hydraulic Retention Time (HRT).

2.3.1.1 Oxygen concentration

Oxygen (O_2) in air is lethal for anaerobic bacteria. If there is O_2 in the environment, anaerobic bacteria paralyze their metabolism and stop developing. The aerobic bacteria use O_2 and release CO_2 in their metabolism and the anaerobic bacteria produce CH_4 as a final product of their metabolism [9].

While CH_4 is a gas rich in chemical energy and therefore can be used as fuel, CO_2 is already fully oxidized and can't be used as fuel. If the digester is not airtight, biogas quality will decrease because anaerobic bacteria die and the aerobic ones survive, the biogas will then be rich in CO_2, instead of CH_4. Thus, the digester must provide a complete tightness to

cause a complete lack of O_2 inside, that is, the complete anaerobic environment needed for the metabolism of anaerobic bacteria.

2.3.1.2 pH

The pH is an important feature for keeping functional anaerobic digestion. A favourable pH is in the range of 5.6-7.6 (Parkin, G., 1986). For the different phases of anaerobic digestion, the optimum pH value changes, being in a range of 5.6-6.0, from hydrolysis to acetogenesis and between 7.2-7.5 in methanogenesis.

The accumulation of intermediate acids can lead to pH drop during fermentation. In order to maintain a stable operation, it is possible to add bicarbonate or carbonate as an alkalinity buffer to neutralize volatile fatty acids and CO_2 (Parkin, G., 1986). Although acetate and ammonia are two alternative buffers for anaerobic systems, these chemicals are unlikely to provide sufficient buffering capacity in AD systems. Inhibition on AD occurs while pH value is too high or too low.

2.3.1.3 Temperature

Since the speed of reaction is the phenomenon governing the process, the temperature becomes a very important parameter. The mixture to be digested must be brought to the temperature required by the particular process used.

The effects of temperature on AD are well recognized. The reaction efficiency depends directly from the biological activity of the microorganisms inside the reactor.

There are three groups of bacteria classified in terms of operating temperature: Psicrophilic, Mesophilic and Termophilic.

The rate of anaerobic degradation of organic substrates generally increases in the order of psychrophilic, mesophilic and thermophilic digestion.

Psicrophilic microorganisms operate in optimum conditions for temperatures between 15-18ºC (if obliged) or 25-30ºC (if facultative), not operating so well for temperatures inferior to -5ºC and superior to 22ºC (if obliged) or 35ºC (if facultative).

Mesophilic microorganisms operate in optimum conditions for temperatures between 30-45ºC, not operating below 10ºC and above 47ºC.

Termophilic microorganisms operate in optimum conditions for temperatures between 55-75ºC, not operating for temperatures below 40ºC and above 80ºC.

Suddenly changes of only 5ºC, produce considerable decreases of the productive efficiency. Most full-scale anaerobic digesters are operated at mesophilic temperature (Parkin,

G., 1986), and to optimize the digestion process, the biodigester must be kept at a constant temperature, as rapid changes will upset bacterial activity.

2.3.1.4 Substrate composition

The characteristic of the input substrate affects the biogas yield productivity, by affecting directly the microorganisms responsible for the biogas production.

Knowing this fact it is important to analyze previously the physical characteristics (straw and fiber), chemical (substances toxic for bacteria) and the presence of antibiotics, disinfectants, detergent in the biomass that will be inputted in the reactors, because they can slow down or inhibit the process [9].

The input material must contain adequate levels of organic matter and elements essential to growth and multiplication of microorganisms involved in the process.

The table 2 shows some ions that interact with the biogas production, the optimal concentrations and the concentrations that inhibit the process.

Table 2 - Optimal concentrations of ions in the slurry and inhibition concentrations.

Inorganic ions		Optimal concentration	Low inhibition	Strong inhibition
Sodium	(mg/L)	100-200	3500-5500	8000
Potassium	(mg/L)	200-400	2500-4500	12000
Calcium	(mg/L)	100-400	2500-4500	8000
Magnesium	(mg/L)	75-150	1000-1500	3000
Ammonium	(mg/L)	50-1000	1500	8000
Sulfide	(mg/L)	0,1-10	100	200
Chromium	(% per solids)	-	2	3
Cobalt	(mg/L)	20	-	-
Cupper	(mg/L)	-	-	200

Other factors affecting the rate and amount of biogas output are: moister, carbon/nitrogen ratio (C/N), mixing of the digesting material and the particle size of the material being digested. It may be necessary to add co-substrate to the feed material if it is too dry, or if the nitrogen content is very high, a C/N ratio of 20/1 to 30/1 is best, occasional mixing or agitation of the digesting material can aid the digestion process[9]. If this ratio takes very high values, the process develops slowly. If the values are too low, there may be an overproduction of ammonia that, at concentrations higher than 3 g/L, is toxic to methanogenic bacteria.

Antibiotics in livestock feed have been known to kill the anaerobic bacteria in

digesters, since they can still be present in the livestock manure. The action of microorganisms is the more effective the higher is the specific surface of the particles of various materials constituting the substrate, the particle size of biomass to be treated should be as thin as possible. Pre-sizing and mixing of the feed material for a uniform consistency allows the bacteria to work quicker.

2.3.1.5 Hydraulic Retention Time

Hydraulic retention time (HRT) is the time that the OM remains in the digester and is calculated by equation 1:

$$\boldsymbol{HRT} = \frac{\boldsymbol{V}}{\boldsymbol{Q}} \qquad (1)$$

Where Q (m^3/day) is the volume of material which has to be digested per day and V (m^3) is the volume of digester. In traditional plants which operate in mesophilic conditions (30-40ºC), HRT = 10-30 days.

Since AD depends on the biological activity of relatively slowly reproducing methanogenic bacteria, these bacteria must be given sufficient time to reproduce, so that they can replace cells lost with the effluent sludge, and adjust their population size to follow fluctuations in organic loading (if is that the case). If the rate of bacteria lost from the digester with the effluent slurry exceeds the growth rate of the bacteria, the bacterial population in the digester will be “washed out" of the system. This washout is avoided by maintaining a sufficient retention time for solids ensuring that the bacterial cells remain in optimal concentration within the digester (VDI 4630, 2006).

Operational conditions, referred above, which influence biological reactions, such as pH, temperature, inhibitors (antibiotics, detergents) concentrations, are amenable to external control in the anaerobic digestion process.

2.4 Biogas

Biogas is mixture of approximately 35% to 70% CH_4, 25% to 60% CO_2, and small amounts of nitrogen (N_2, 4%), hydrogen (H_2, 1%,) hydrogen sulphide (H_2S, 2%), water vapor and other gases, and can be used as a biofuel to produce energy. CH_4 is the simplest hydrocarbon, is an odorless and colorless gas and have a calorific power of 25-39.8 MJ/m^3, the possible smell comes from the other gases that composes the biogas, like hydrogen sulphide. CH_4 is the gas that gives more energy properties to biogas, presenting itself as a potential renewable biofuel.

The quantities of CH_4 and other compounds depended on the type of input materials used. Biogas is similar to a gas that can be obtained from oil refining (natural gas) and can be naturally found in swamps.

The CH_4 in biogas can be burned to produce both heat and electricity, usually using CHP technology in a cogeneration arrangement where the electricity and wasted heat generated are used to warm the digesters or to heat buildings. Excess electricity can be sold to suppliers or put into the local grid. Electricity produced by anaerobic digesters is considered to be renewable energy.

Biogas conversion into energy does not contribute to increasing atmospheric GHG concentrations because the CH_4 is burned and the CO_2 expelled to the atmosphere comes from an organic source.

The energy produced from 1 m^3 of biogas is energetically equivalent to 1.5 m^3 of gas for cooking; 0.8 liters of gasoline; 0.7 liters of diesel; 1.3 liters of alcohol; 7 kWh of electricity.

According to Giraldi, D. et al. (2008), biogas production from maize stalks is around 300 – 700 L_N/kg_{VS}, maize grain biogas yield is around 400 $L_N/kg_{VS,}$ and maize (whole crop) is around 380 L_N/kg_{VS}[3].

2.5 Anaerobic Digestion in Europe

Many countries in Europe that have not yet increased anaerobic digestion practice to produce biogas are predisposed to take advantage of biogas. In Italy, for example, cars running on natural gas or on both natural gas and petrol are widespread.

Germany and Austria also have cars already running on natural gas, and have both gone into biogas enthusiastically, though mostly using bioenergy crops as feedstock. They recently set up national targets of 20% biogas in the gas sold to vehicles.

At the end of 2006, Germany had about 3 500 biogas plants with total electric capacity of 1.1 GW in operation. Most of the new biogas plants have an electrical capacity between 400 – 800 kW. The first industrial biogas energy park, Klarsee, with 40 biogas plants (total capacity 20 MW, has come into operation. Energy crops are the main substrate, and manure constitutes less than 50%. Industrial companies mainly built plants for fermentation of energy crops. Germany is already growing energy crops on more than 1.3 million ha, or 11.4% of its arable land. Currently, there are quite a few large biogas digesters at wastewater treatment plants, landfill gas installations, and industrial bio-waste processing facilities, and more are under construction. But it has been predicted that by 2020, the largest volume of produced biogas will come from farms and large co-digestion biogas plants, integrated into the farming and food-processing structures [7].

How much biogas energy can we realistically expect for Europe as a whole, counting both energy crops and livestock manure? One estimate from the University of Southern Denmark assumed that energy crops convert to biogas at an efficiency of 80%, as not all the compounds from biomass can be digested, for example lignin, and only around 25% of the energy crop will be dedicated for biogas production, the rest to be applied to other renewable energy production such as solid and liquid biofuels. The EU27 has a total land area of 433.2 Mha, of which 196.6 Mha are agricultural and 113.5 Mha are arable. If 20% of arable land is dedicated to energy crops such as switch grass – so 5% goes to biogas - 45.5 Mtoe (megatonne of oil equivalent) of CH_4 can be produced at a projected yield of 20 tonnes of solids/ha, about twice as high as currently achievable.

In addition, the EU27 produces 1 578 Mt of cow and pig manure a year. The animal production sector is responsible for 18% of the GHG emissions, which includes 37% of the anthropogenic CH_4 and 65% of anthropogenic nitrous oxide. The total potential for CH_4 from the livestock manure is 18.5 Mtoe. Hence, a total of 64 Mtoe, or 71 200 million m^3 of CH_4 can be produced by 2020 from energy crops grown on 5% of Europe's arable land, plus its great quantities of livestock manure. This does not quite make up for the 74 400 million m^3 of natural gas CH_4 that EU currently imports from Russia.

Obviously, if all the energy crops on 20% of EU-27's arable land were to be converted into biogas/ CH_4 – which makes sense as it is far more efficient than conversion into ethanol or biodiesel - the estimates improve by quite a lot, as it would yield 182 Mtoe, giving a total of 200.5 Mtoe, about 10% of the current EU energy consumption of about 2 Gtoe.

Natural gas consumption has increased in the last 30 years and now accounts for almost one quarter of the world's energy consumption. It is projected to account for 43% by 2030. The theoretical potential of biogas CH_4 in EU27 would produce enough to supply 15.5% of the natural gas consumption in Europe (or considerably more if all energy crops were dedicated to biogas CH_4 production). At the same time, the emissions of several toxic compounds like nitrogen oxides and reactive hydrocarbon can be reduced by up to 80% compared to petrol and diesel.

A big question mark is whether dedicating 20% of Europe's arable land to producing energy crops is sustainable in terms of food production and conservation of natural biodiversity. Practically all of the set-aside land would have to be pressed into crop production.

2.6 Anaerobic Digestion in Portugal

The implementation of anaerobic digestion systems and use of biogas in Portugal over the past 35 years reveals a wide range of popularity. During the 70's until the 80's, there was a need to fix the environmental problems in agriculture and livestock areas and there was a lot of government money to finance new projects, so anaerobic digestion was a popular technology to solve those problems.

The quality of the constructed facilities was low. In the initial phase of implementation and experimentation – some negative results occurred, causing bad reputation and dissent regarding anaerobic digestion technologies and their profitability.

In the 90's, a set of circumstances, such as the introduction of natural gas, the subsidies reduction, the permissive environmental laws promulgation on the discharge of agro-livestock effluents and other specific technical issues (businesses location, micro-production, etc…) discouraged the application of anaerobic digestion as a effluents treatment from agriculture and blocked for several years, the development of centralized systems of anaerobic digestion, which was very popular in EU.

However, few years ago, the need to provide solutions to the environmental problems, has originated the implementation of centralized solutions to agro-livestock residues, following the models from the 80's and including an advanced debugging of the digested products. In the area of municipal wastewater and sludge treatment in the last 20 years numerous Wastewater treatment plants have been implemented to serve more than 80% of the population. The conventional biological treatments applied to the sewage, does not destroy the organic matter, but only transfer most of the pollutant compounds in the form of sludge, producing daily large quantities, which treatment, management and final destination is a serious problem. Nowadays, some of the big Wastewater Treatment plants have an anaerobic digestion system to treat the sludge, producing biogas, which is converted to energy and sold to the network. The digested sludge has good fertilizer properties, but first needs to be stabilized and hygienized. The next phase is to extend this solution to small and medium scale plants, to increase the biogas production and use.

Since the year 2000 in Portugal, some municipal solid wastes are properly health and environmentally controlled. In regions with high population density, incineration has been the preferred solution, while landfill disposal is the most popular, being applied to a population equivalent of 6.8 million inhabitants. This solution will be gradually replaced by anaerobic digestion and recycling systems in order to meet the limits set by 1999/31/EU Directive, which restricts the biodegradable organic matter disposal in landfills. The Portuguese plan PERSU II specifies the objectives, now clearly favorable on anaerobic digestion.

In the short term it is expected 10 to 12 digesters are built for the treatment of urban

municipal solid wastes which can include co-digestion of any other wastes available locally. It is observed that there was a real change in mentality in this context. Anaerobic digestion has already increased acceptance and is already recognized as an important tool to achieve environmental and energy objectives of the country and is also a potential area of business, representing 0.5 % of the Renewable energy source (RES) produced in 2011 (DGEG, 2011).

2.7 Environmental sustainability of AD

The concept of sustainability has a lot of different definitions. The most popular definition of sustainability can be traced to a 1987 UN conference. It defined sustainable developments as those that "meet present needs without compromising the ability of future generations to meet their needs" (WECD, 1987).

This well-established definition set an ideal premise, but doesn't clarify specific human and environmental parameters for modeling and measuring sustainable developments.

The following definition is more specific: "Sustainable developments are those which fulfill present and future needs" (WECD, 1987) "while [only] using and not harming renewable resources and unique human-environmental systems of a site: [air], water, land, energy, and human ecology and/or those of other [off-site] sustainable systems" (Rosenbaum, 1993 and Vieira, 1993).

AD is environmental sustainable because: reduces the amount of GHG, collecting CH_4 (23 times more strong as a GHG than CO_2) and providing a source of renewable energy that is carbon neutral i.e. provides energy with no net increase in atmospheric CO_2; reduces the odor from farm slurries by up to 80%; use biomass that is hard to give a final destination, such as livestock manure and slurry; low the BOD, (a measure of the polluting strength of a material) in the feedstock to less than 40% of that in the digestate; low the pathogens concentration in the feedstock, such as salmonella; have better management of nitrate and agronomic/energetic value (the nitrogen in digestate is more readily available as a plant nutrient, can be used as fertilizer, herbicide, it fibers can act as a soil conditioner and can be dried and used as combustible fuel) [1].

3. Materials and Methods

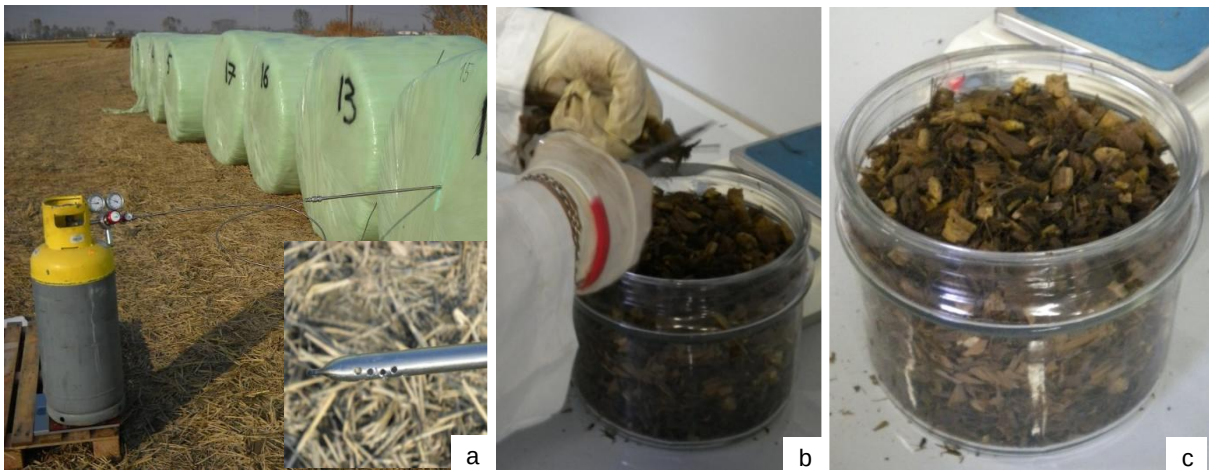
This experience was developed between May 2011 and July 2011, in the laboratories of DEIAFA (Dipartimento di Economia e Ingegneria Agraria Forestale e Ambientale) in the Università degli Studi di Torino – Facultà di Agraria, Italy.

Maize straw used in the trials was from a hybrid named *Pioneer 130*, which was cropped, wrapped and collected in a farm called “Azienda agricola Berardo Giovanni” located in Borgo Vercelli, Italy. The unwrapped sample was collected on the 20th October 2010 and the ones with ammonia were pretreated on the 24th November 2010 and collected on the 11th May 2011.

Ammonia pretreatment consisted in injecting gaseous ammonia by a spray gun directly in the bales (picture 9a) at the rate of 1%, 1.5%, 2% and 4%. The amount of ammonia needed to reach the target concentrations was calculated according to the amount of TS of each maize stalks bale. It is a procedure that requires a lot of time and it is impossible to apply all the bales in the same day, which is bad, because environmental conditions can change and there is a difference between the bales related to the time in contact with ammonia.

Mechanic pretreatment was made for every different biomass in duplicate, using scissors, reducing the stalks size to dimensions approximately to 1 cm (pictures 9b and 9c).

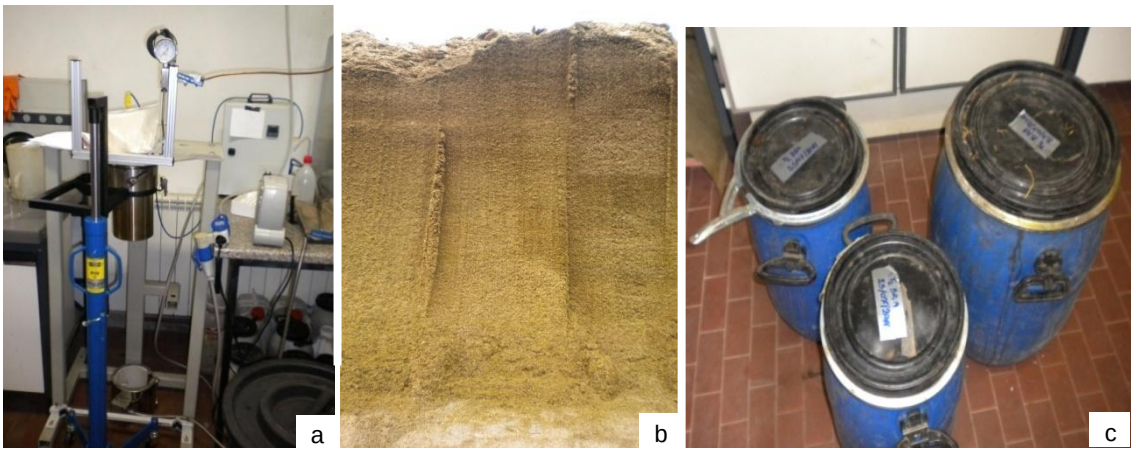
Thermal pretreatment was made using a special autoclave at ±120 ºC for 30 minutes (picture 10a), and the Mechanic + Thermal pretreatment was the combination of those two techniques described before.



Picture 9 – a) Chemical pretreatment application with spray gun; b**)** Mechanic pretreatment; **c)** Final result of mechanic pretreatment.

Digestate samples used as inoculum were collected form a CSTR (Continuous Stirred Tank Reactor) Biogas plant called “La Speranza”, located in Candiolo (Piemonte region of northwestern Italy). This biogas plant was fed with animal effluents and energy crops at different ratios, in particular, solid materials (triticale and maize silage, manure and separated solid fraction) (picture 10b) plus slurry.

All samples were collected at the exit of the post-fermenter and before their inlet into the storage tank. Samples were stored in gas-tight containers for transport to the lab (picture 10c). Digestate had 4.13% of TS, 2.89% of VS per fresh material and 69.70% of VS per TS.



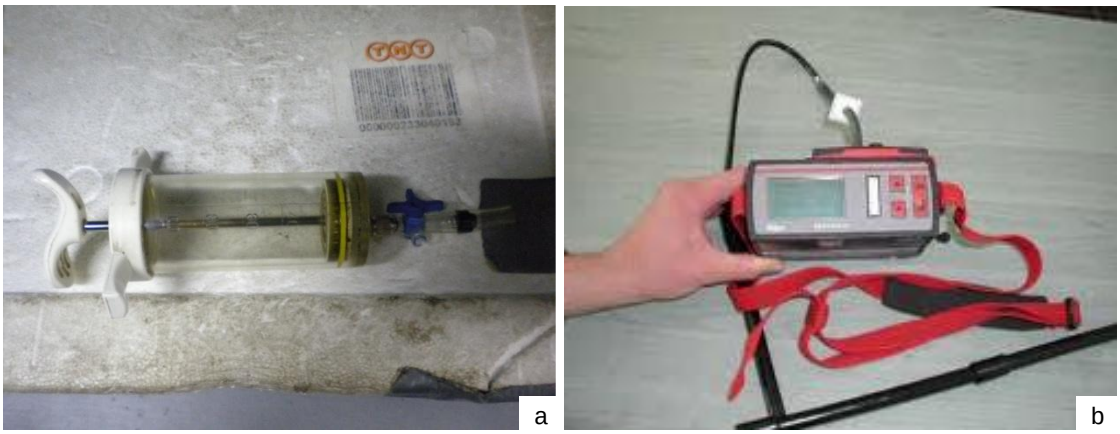
Picture 10 – a) Autoclave used for thermal pretreatment; **b)** Pile of biomass to fuel biogas plant; **c)** Digestate's transport vessels.

The trials were performed in 2 L anaerobic batch reactors, according to Verein Deutscher Ingenieure (VDI) 4630 (2006) and following the experimental procedure described by Dinuccio et al. (2010). Specifically, at the beginning of the trials, each reactor was filled with approximately 1200g of digestate, 300g of deionized water and the corresponding amount of fresh biomass (based on VS values and obeying to a 0,5 ratio of inoculum per OM from biomass), like in picture 11a and 11b and sealed with glass taps connected to Aluminum gas bags.

Picture 11 – a) Batch filling sequence; **b)** Set of full batches ready to tap; **c)** Batches in the temperature controlled room 1, already with aluminum gas bags attached.

Samples were incubated at 40 ºC (±1 ºC) in two temperature controlled rooms for 50 days (picture 11c and 12a). All reactors were manually stirred twice a day. Each trial and control test (just inoculum and water) were performed in three replicates resulting in a total of 75 samples. Biogas was regularly measured with a drum-type gas meter (Ritter TG05/5, Bochum, Germany) (picture 12b) and analyzed CH_4 content by an infrared analyzer (Dräger Multiwarn) (picture 13b). To prevent operating errors, the CH_4 readings were preformed three times for each bag, collecting (when possible) 100 mL each time with a syringe (picture 13a).

Picture 12 – a) Batches in the temperature controlled room 2, already with aluminum gas bags attached; **b)** Gas meter Ritter TG05/5.



Picture 13 – a) Syringe to sampling CH_4 from aluminum gas bags; **b)** Infrared device used to read CH_4 concentration, Dräger Multiwarn.

The recorded data were normalized at standard temperature and pressure (0 ºC and 1013 hPa) and to normal liters (L_N) according to German Standard Procedure (VDI 4630, 2006), using the following equation:

$$\boldsymbol{V_0^{tr}} = \boldsymbol{V}.\frac{(\boldsymbol{p}-\boldsymbol{p_w}).\boldsymbol{T_0}}{\boldsymbol{p_0}.\boldsymbol{T}} \qquad (2)$$

Where:

$\boldsymbol{V_0^{tr}}$ - Volume of the dry gas in the normal state, in mL_N.

$\boldsymbol{V}$ - Volume of the gas was readed, in mL.

$\boldsymbol{p}$ - Pressure of the gas phase at the time of reading, in hPa.

$\boldsymbol{p_w}$ - Vapor pressure of the water as a function of the temperature of the ambient space, in hPa.

$\boldsymbol{T_0}$ - Normal temperature; T_0 = 273 K.

$\boldsymbol{p_0}$ - Normal pressure; p_0 = 1013 hPa.

$\boldsymbol{T}$- Temperature of the fermentation gas or of the ambient space, in K.

Since reactors were not completely filled, the normalized volumes of biogas and CH_4 were corrected with the equation reported by VDI 4630 (2006), which is:

$$\boldsymbol{C_{korr}}^{tr} = \boldsymbol{C_{t_2}}^{tr} + (\boldsymbol{C_{t_2}}^{tr} - \boldsymbol{C_{t_1}}^{tr}) \times \frac{\boldsymbol{V_k}}{\boldsymbol{V_b}} \qquad (3)$$

Where:

$\boldsymbol{C_{korr}}^{tr}$ - Is the correct concentration of the biogas components in the dry gas, in % by volume.

$\boldsymbol{C}^{tr}$ - Is the measured concentration of biogas components in the dry gas, in % by volume.

$\boldsymbol{V_k}$ - Is the headspace volume, in mL.

$\boldsymbol{V_b}$ – Volume of the biogas produced, in mL.

t – Time of measurement ($t_2 > t_1$).

At the beginning of the trials, all the samples pretreated were analyzed for TS, VS, C/N ratio, neutral detergent fibers (NDF), acid detergent fibers (ADF) and acid detergent lignin (ADL). From the analysis of these data it can be calculated the content of hemicellulose (HEM) and cellulose (CELL) by subtracting NDF and ADF, and ADF and ADL, respectively. TS were determined after 24h at 105 ºC, VS were determined after 4h at 550 ºC in a muffle furnace (AOAC, 2000). Total nitrogen was determined by Kjeldahl instrument after total mineralization. NDF, ADF and ADL were determined by Van Soest methods (Van Soest et al., 1991).

Net biogas and CH_4 yield from maize straw biomass digestion were calculated by subtracting the volume yield from the blank test, and statistically analyzed with analysis of variance (ANOVA) test, after confirming that the variances were homogeneous by the Levene test, and Tukey's means grouping test (α= 0,05). Also Pearson's correlation coefficient "r" was preformed to evaluate the relationship between the main chemical parameters analyzed in the beginning of the trials and biogas and CH_4 production.

4. Results and discussion

4.1 Chemical characterization

The data collected from periodic analyses of biogas and CH_4 production for each batch were recorded on the Excel worksheets in order to obtain real-time values updates. In picture 15, shows an Excel template used to record data and to do calculations.

With these calculations were obtained for each trial, the mL_{Biogas}/g_{VS} and $mL_{Methane}/g_{VS}$ daily and accumulated, already normalized, and the correspondent graphics. For mean values have been subtracted the shares of biogas and CH_4 from inoculum, in order to obtain, for each trial, the net biogas and CH_4 yield just from biomass degradation. This procedure was only feasible in the end of the trials, because it was not always possible to read inoculum's biogas and CH_4 concentrations at the same time as the other samples due to the low progress yield from inoculum.

Table 3 - Main chemical parameters for feedstock with no pretreatments and mechanically pretreated.

Raw and Mechanic pretreatment										
Biomass	**TS %**	**VS % TS**	**NH_4-N %**	**N_{total} %**	**C % TS**	**C/N % TS**	**NDF % TS**	**LIGN/NDF % TS**	**HEM/NDF % TS**	**CELL/NDF % TS**
MSU	38.60	84.34	0.01	0.26	14.00	53.80	78.24	0.12	0.38	0.50
MSW	39.26	79.95	0.02	0.30	14.59	48.60	76.09	0.19	0.29	0.53
MS1	39.98	85.84	0.86	1.44	13.32	9.20	63.85	0.13	0.32	0.56
MS1.5	37.53	84.17	0.76	1.37	11.88	8.70	67.18	0.13	0.33	0.54
MS2	37.36	82.82	1.09	1.64	12.41	7.60	61.63	0.14	0.34	0.52
MS4	33.14	80.66	0.98	1.65	10.89	6.60	49.53	0.12	0.31	0.57

Table 4– Main chemical parameters for feedstock thermally pretreated.

Thermal pretreatment										
Biomass	**TS %**	**VS % TS**	**NH_4-N %**	**N_{total} %**	**C % TS**	**C/N % TS**	**NDF % TS**	**LIGN/NDF % TS**	**HEM/NDF % TS**	**CELL/NDF % TS**
MSU	30.66	86.09	0.03	0.17	10.20	60.00	73.70	0.14	0.31	0.55
MSW	30.03	86.06	0.01	0.32	8.34	26.10	75.26	0.09	0.39	0.52
MS1	30.72	86.31	0.62	0.88	10.68	12.10	76.91	0.14	0.29	0.56
MS1.5	35.99	87.68	0.68	1.28	12.22	9.50	76.91	0.13	0.25	0.62
MS2	42.05	91.91	0.62	1.23	16.10	13.10	67.20	0.17	0.25	0.58
MS4	36.10	79.08	0.85	1.46	13.15	9.00	39.45	0.15	0.33	0.51

Table 5– Main chemical parameters for feedstock mechanically and thermally pretreated.

Mechanic + Thermal pretreatment										
Biomass	**TS %**	**VS % TS**	**NH_4-N %**	**N_{total} %**	**C % TS**	**C/N % TS**	**NDF % TS**	**LIGN/NDF % TS**	**HEM/NDF % TS**	**CELL/NDF % TS**
MSU	20.51	85.90	0.01	0.17	8.00	47.00	66.94	0.10	0.31	0.59
MSW	31.14	86.17	0.02	0.20	11.82	59.10	66.78	0.12	0.31	0.58
MS1	37.63	81.48	0.83	1.31	12.96	9.90	58.70	0.12	0.32	0.56
MS1.5	28.94	81.05	0.63	1.08	9.66	8.90	65.52	0.09	0.39	0.52
MS2	33.73	86.24	0.59	1.07	11.76	11.00	57.27	0.13	0.23	0.63
MS4	35.17	77.13	0.91	1.55	10.15	6.50	44.60	0.18	0.20	0.61

For simplicity reasons, the names of feedstock and pretreatments were changed. MSU, MSW, MS1, MS1.5, MS2 and MS4 correspond, in order to maize stalks Unwrapped, Wrapped, 1%, 1.5%, 2% and 4% NH_3 and pretreatments Raw, Mec, TH and Mec+TH, correspond, in order to no pretreatment done, mechanically pretreated, thermally pretreated and mechanically and thermally pretreated.

On tables 3, 4 and 5 are the main chemical feedstock parameters. Since on mechanic pretreatment was simply done size reduction, which implies that the feedstock are the same as the ones not pretreated, so the main chemical feedstock parameters are the same. The discussion of those results is made considering the tables 3, 4 and 5 as a single table.

The content in TS represents the quantity of dry material (organic and inorganic) and varies between 42.05% (MS2 TH) and 20.51% (MSU Mec+TH) on Maize stalk residues. These values are related with humidity biomass content, notice that TS values decrease when thermal pretreatment is done. Since thermal pretreatment requires additional water to be done and comparing the highest value with the others from the same pretreatment, maybe it was used too much water and this value is not so correct as it was expected, maybe the highest value should be 39.98% (MS1 None/Mec). Since the different thermally pretreatments were done at different times, the anomalous value can be just an operating error.

As expected, ammoniacal nitrogen (NH_4-N) content is higher on samples chemically pretreated with higher ammonia concentrations, like in MS1, MS1.5, MS2 and MS4 feedstock in all the different pretreatments, and it ranges between 0.01% (MSU Raw/Mec, MSW TH and MSW Mec+TH) and 1.09% (MS2 None/Mec).

The total nitrogen (N_{total}) is similar to NH_4-N, in the terms that samples chemically pretreated with higher ammonia concentrations have higher N_{total} content, but it is noticeable a difference between MSU and MSW feedstock N_{total} content, where MSW have higher values than MSU. This difference is explained due the fact that MSW biomass bales were wrapped

in plastic, preventing volatile N to escape to the atmosphere and MSU biomass bales were not. N_{total} decreases can also mean biological activity. The content in N_{total} ranges between 0.17% (MSU Mec+TH and MSU TH) and 1.65% (MS4 Raw/Mec).

Carbon (C) content do not show significant differences between feedstock or pre-treatments, ranging its content between 8% on TS (MSU Mec+TH) and 16.10% on TS (MS2 TH).

C/N ratio ranges between 6.5% (MS4 Mec+TH) and 60% on TS (MSU TH) and there is a particular difference on C/N ratio values between ammonia pretreated feedstock and not chemically pretreated feedstock, where chemically pretreated have lower values, due to their high N content. Particular high C/N ratio on feedstock before anaerobic digestion is not such a big problem, considering their subsequent co-digestion with inoculum, the nitrogen content in the digested slurry can help to adjust the ratio to ideal levels.

On the opposite, when C/N ratio is already low, anaerobic digestion will lower this value, moving it away from the optimal conditions, which are C/N ratios between 20-30%.

NDF content is the amount of structural carbohydrate in biomass and includes digestible (hemicellulose), less digestible (cellulose) and indigestible (lignin) components. The highest value (78.24% on TS) was obtained in MSU Raw/Mec biomass and the lowest (39.45) on MS2 Mec+TH biomass. It is noticeable a decrease, analyzing for biomass type from MSU for MS4, except for MS2, which had an erratic behavior. Higher values belong to Raw/Mec pretreatments and lower values belong to Mec+TH pretreatment, being obvious the effect of TH and Mec+TH pretreatments on NDF levels.

Since lignin, hemicellulose and cellulose values are related to NDF content, it can obtained their content on NDF, by dividing each one of them by NDF content. Those ratios are represented in tables 3, 4 and 5 on LIGN/NDF, HEMI/NDF and CELL/NDF columns.

Lower lignin levels and higher cellulose levels were found on Mec+TH pretreatment, which is logic, since mechanical pretreatment increases the accessible surface area and size of pores, and decrease the crystallinity and degrees of polymerization of cellulose by reducing the biomass size (Angelidaki, I. and Ahring, B., 2000) and thermal pretreatment causes the disruption of cell walls, releasing it content, without sugar degradation.

The lowest hemicellulose values were found on the feedstock thermally pretreated, and these losses are higher, when higher is the concentration of the chemical pretreatment, indicating that part of hemicellulose is solubilized during thermal pretreatment. The evident difference on chemically pretreated may be due the fact that the delignifying effect of NH_3 on hemicellulose made it more susceptible to thermal pretreatment, because is less “protected”.

4.2 Biogas and methane production from Maize crop residues

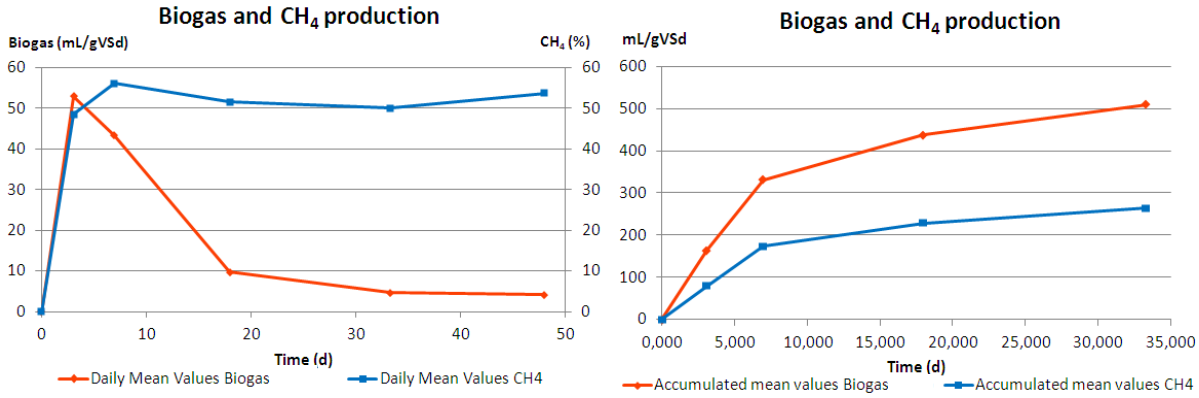
From the measuring of the aluminum bags, it was possible to analyze the biogas and CH_4 raw production and construct the performance curves that describe the biogas and CH_4 yield.

The performance curves are typical of biogas productions in a batch reactor. For this reason, the biogas curve should ideally be comparable to a bell curve with positive asymmetry. After an initial peak, there is a slow and continuous decrease in volume until it reaches zero, duo the fact that substrate is not immediately available to be degraded. The performance curves depend on substrate degradation rate: readily convertible substrates have performance curves with an immediate increase in the amount of biogas produced, while substances with difficult degradation, like lignocellulosic feedstock, show a delay in biogas production in their performance curves (VDI 4630, 2006).

The curve obtained from the observations of the cumulative CH_4 production, it should ideally correspond to a sigmoid, with a progressive exponential growth over the first 7-10 days, then slow down and stay with a linear trend, around values between 50-60% until the exhaustion of the substrate. As the biogas curve, the appearance of the initial peak depends on biomass degradability.

In picture 14, the curves obtained in the experimental procedure for biogas and CH_4 concentration, correspond approximately to the ideals. Not all the trials were as good as this one, but generally the pattern of the curve was similar.

The accumulation curve is always increasing, as expected, since it accumulates the daily mean values, stabilizing when the biogas and CH_4 production slows or stop.



Picture 14 - Performance curves of "daily" mean values of raw biogas and CH_4 composition and accumulative mean values of raw biogas and CH_4 production (MS2 Mec).

Sample	Date	Time	Δt0	V_1	V_2	ADJ	ΔV	ΣV	CH_4 per tq	T	Pw	NV	CH_4 per dry	CH_4 def	CH_4	$TS_{biomass}$	$VS_{biomass}$
1			[h]	[L]	[L]	(L)	[L]	[L]	[%]	°C	hPa	L	[%]	[%]	[L]	[g]	[g]
0	8-6-11	18:00	0:00:00					0,000					0,00	0,00	0,000		
A	12-6-11	13:00	91:00:00	27479,816	27485,530	0,270	5,984	5,984	41,4	22	25,28	5,40	42,46	45,88	2,477	44,85	16,76
B	17-6-11	15:45	213:45:00	27645,546	27647,112	0,380	1,946	7,930	54,7	24	28,46	1,74	56,25	59,69	1,038	44,85	16,76
C	23-6-11	17:00	359:00:00	27747,398	27748,768	0,240	1,610	9,540	51,0	24	28,46	1,44	52,47	51,33	0,738	44,85	16,76
D	8-7-11	10:30	712:30:00	28000,282	28001,466	0,360	1,544	11,084	47,0	26	32,01	1,37	48,53	47,28	0,645	44,85	16,76
E	26-7-11	11:00	1145:00:00	28136,494	28137,008	0,260	0,774	11,858	37,7	24	28,46	0,69	38,79	32,66	0,226	44,85	16,76
		Max	1145:00:00								Total	10,63		Total	5,125		

Note: Decimal fraction is comma separated; **A – E** is the nomenclature of the quantity of gas bags that were read; **V_1** and **V_2** are the readings on the gas meter before and after reading; **ADJ** is the volume left in the bag plus the mL taken with the syringe; **NV** is the normalized biogas volume (correspond to $\mathbf{V_0^{tr}}$ from equation 2); **CH4 per tq** is the percentage of methane reading from Dräger Multiwarn per fresh material; **CH_4 def** is the normalized CH_4 (correspond to $\mathbf{C_{korr}}^{\mathbf{tr}}$ from equation 3).

$TS_{inoculum}$	$VS_{inoculum}$	time	time	Biogas brute							CH_4 brute					
[g]	[g]	[Δt(t-1)]	[Σday]	[mL]	[ΣmL]	[mL/d]	[mL/gVSd]	[ΣmL/gVSd]	[mL/gVS]	[ΣmL/gVS]	[mL]	[ΣmL]	[mL/d]	[mL/gVSd]	[ΣmL/gVSd]	[ΣmL/gVS]
			0,000		0,000		0,000	0,000	0,000	0,000		0,000		0,000	0,000	0,000
49,89	34,77	3,792	3,792	5399,548	5399,548	1424,057	40,954	40,954	322,217	322,217	2477,194	2477,194	653,326	38,987	38,987	147,826
49,89	34,77	5,115	8,906	1738,499	7138,047	339,910	9,775	50,729	103,745	425,962	1037,782	3514,976	202,906	12,108	51,095	209,756
49,89	34,77	6,052	14,958	1438,326	8576,373	237,658	6,835	57,564	85,832	511,793	738,349	4253,324	121,999	7,280	58,376	253,816
49,89	34,77	14,729	29,688	1365,193	9941,567	92,686	2,666	60,230	81,468	593,261	645,448	4898,773	43,821	2,615	60,991	292,333
49,89	34,77	18,021	47,708	691,469	10633,035	38,371	1,103	61,333	41,263	634,524	225,861	5124,634	12,533	0,748	61,739	305,812
									Max	634,524					Max	305,812

Picture 15 - Excel worksheet example.

The following tables (6 and 7) refer to net biogas and CH_4 production. The values of biogas and CH_4 were calculated by accumulating the daily productions in function of their VS content and to obtain the net production, the inoculum yields were subtracted, as it shows in the equation 3 and 4.

$$\mathbf{Biogas_{corr} = [\Sigma biogas - (biogas_{inoculum} * VS_{inoculum})]/VS_{biomass}} \quad (3)$$

$$\mathbf{CH_{4\,corr} = [\Sigma CH_4 - (CH_{4\,inoculum} * VS_{inoculum})]/VS_{biomass}} \quad (4)$$

Were:

$Biogas_{corr}$ and **$CH_{4\,corr}$** are the net production in L_N/g_{SV};

Σbiogas and **ΣCH_4** are the raw production, produced in the end of the trial in mL;

$biogas_{inoculum}$ and **$CH_{4\,inoculum}$** are the mL of biogas and CH_4 productoin for g_{SV} of inoculum;

$VS_{inoculum}$ is the grams of VS present in inoculum.

$VS_{biomass}$ is the grams of VS present in biomass.

Table 6 – Mean values of net biogas production in L_N/kg_{VS}.

Biogas net (L_N/kg_{VS})				
Biomass	**Pretreatments**			
	Raw	**Mec**	**TH**	**Mec+TH**
MSU	189.797	180.810	513.920	389.780
MSW	376.162	376.439	405.796	427.021
MS1	376.482	421.349	315.793	248.892
MS1.5	467.570	368.931	315.165	327.061
MS2	550.893	475.068	257.890	273.009
MS4	380.847	426.217	360.128	325.517

Table 7 – Increase of biogas in relation with MSU Raw, in percentage.

Biogas net increase (%)				
Biomass	**Pretreatment**			
	Raw	**Mec**	**TH**	**Mec+TH**
MSU	-	-4.7	170.8	105.4
MSW	98.2	98.3	113.8	125.0
MS1	98.4	122.0	66.4	31.1
MS1.5	146.4	94.4	66.1	72.3
MS2	190.3	150.3	35.9	43.8
MS4	100.7	124.6	89.7	71.5

Table 8 – Mean values of net methane production in L_N/kg_{VS}.

CH_4 net (L_N/kg_{VS})				
Biomass	**Pretreatments**			
	Raw	**Mec**	**TH**	**Mec+TH**
MSU	110.324	82.764	264.201	210.394
MSW	185.229	194.701	199.605	209.415
MS1	208.876	229.574	177.317	140.821
MS1.5	251.985	209.254	173.901	186.318
MS2	280.265	267.662	138.120	144.675
MS4	198.783	242.241	190.458	178.776

Table 9 – Increase of methane in relation with MSU Raw, in percentage.

CH_4 net increase (%)				
Biomass	**Pretreatment**			
	Raw	**Mec**	**TH**	**Mec+TH**
MSU	-	-25.0	139.5	90.7
MSW	67.9	76.5	80.9	89.8
MS1	89.3	108.1	60.7	27.6
MS1.5	128.4	89.7	57.6	68.9
MS2	154.0	142.6	25.2	31.1
MS4	80.2	119.6	72.6	62.0

The CH_4 percentage on biogas varied between 51-72.5%. Analyzing the data of table 6, 7, 8 and 9 and picture 16, it was evident that CH_4 yield had the same trend as biogas yield.

The highest value of biogas and CH_4 were found in MS2 Raw (467.570 L_N/kg_{VS} and 280.265 L_N/kg_{VS}, respectively) and the lowest were found in MSU Mec (180.81 L_N/kg_{VS} and 82.764 L_N/kg_{VS}, respectively). It is noticeable a biogas and CH_4 yields increase on the feedstock pretreated chemically comparing to the ones which were not pretreated chemically, which could be explained by the lignin, hemicellulose and cellulose solubilization. It seems to be that lignin, hemicellulose and cellulose levels are limiting variables, since lower levels, after pretreatment, produces more biogas and CH_4 and higher levels of these compounds, produces less (Ward, A. J. et al., 2008). This increase on biogas and CH_4 yield was verified, only when they were not pretreated thermally. Here it is clear a negative interaction between ammonia and thermal pretreatment.

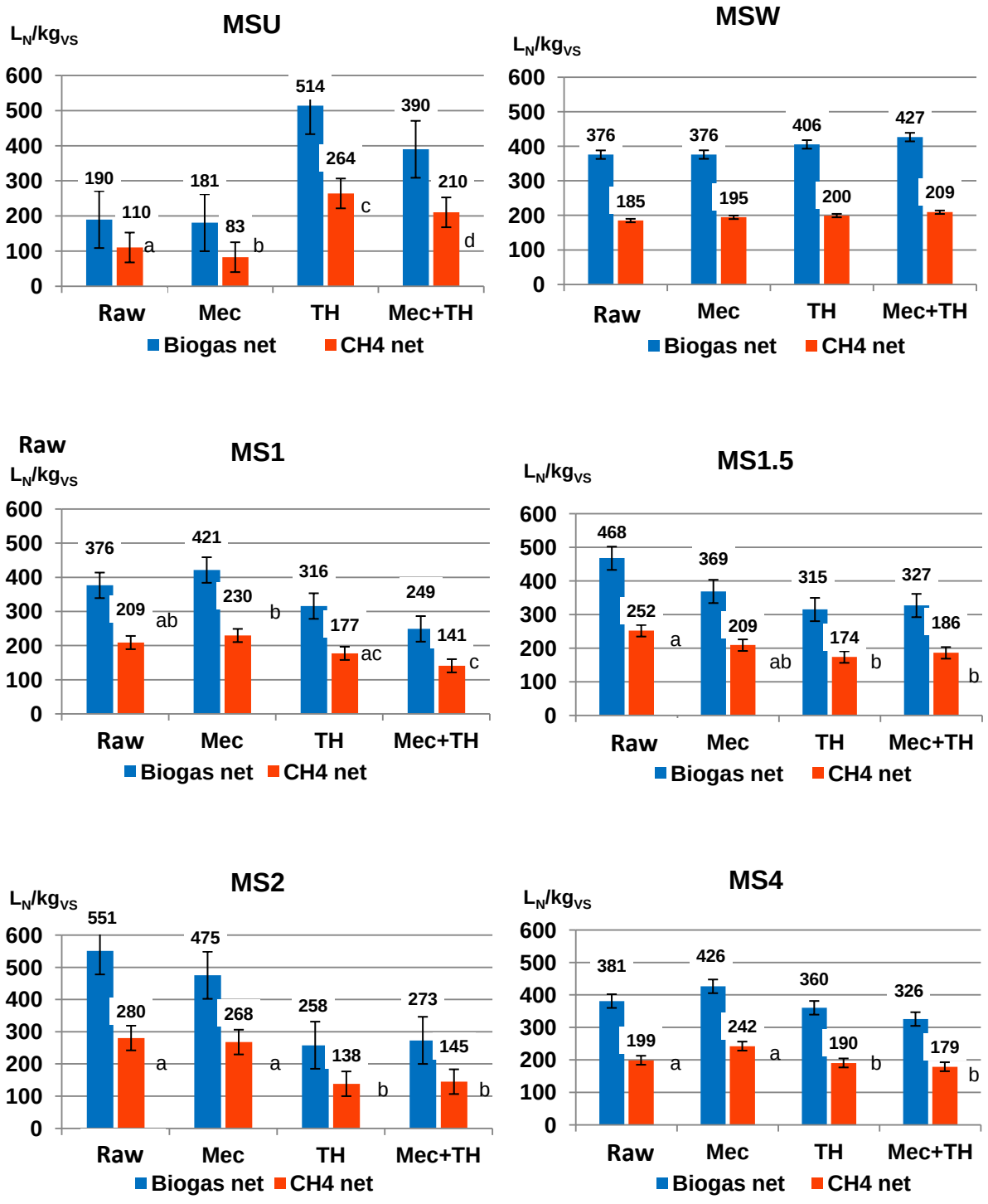
For MSU biomass, the best biogas and CH_4 yields were found in TH pretreatment (427.021 L_N/kg_{VS} and 264.201 L_N/kg_{VS}, respectively) and the lowest in Mec pretreatment (180.81 L_N/kg_{VS} and 82.764 L_N/kg_{VS}, respectively). According to references, the highest result is logic, since TH pretreatment increases the digestible content of biomass (Mladenovska et al., 2006).

For MSW the best biogas and CH_4 yields were found in Mec+TH pretreatment (513.92 L_N/kg_{VS} and 209.415 L_N/kg_{VS}, respectively) and the lowest in Raw pretreatment (376.162 L_N/kg_{VS} and 185.229 L_N/kg_{VS}, respectively). This biomass have an expected behavior, since the values between pretreatments are higher from Raw to Mec+TH, which indicates that pretreat mechanically and thermally this biomass is more effective, in terms of biogas and CH_4 yields, then just applying mechanic or thermal pretreatment.

MS1 and MS4 feedstock present a similar behavior. From Raw to Mec, biogas and CH_4 yields increases, as expected, since Mec pretreatment makes biomass more available to microorganisms (Angelidaki, I. et al., B., 2000), but when the biomass is pretreated thermally, biogas and CH_4 yields decreases.

Like in MS1 and MS4 feedstock, the biogas and CH_4 yields from thermally pretreated feedstock decreases in the MS1.5 and MS2 feedstock, but this time, there is also a decrease from Raw to Mec in biogas and CH_4 yield terms.

As it can be seen in table 7 and 9, in the feedstock pretreated chemically and thermally and for higher NH_3 concentrations, biogas and CH_4 yields shows a decreases when comparing to the other two different pretreatments (None and Mec), were the thermal pretreatment demonstrate the lowest increase. This could be due to the possible digester acidification, oxygen concentration or the formation of inhibitory compounds. The pretreatment which had increased more the biogas production was Raw pretreatment.



Picture 16 - Graphics of net biogas and methane for each biomass. Error bars indicate standard error (N = 3). Means followed by the same letter are not statistically different using Tukey's test at 5% level

4.3 Statistical analysis

Analyzing Person's correlation coefficient "r" for all the trial, there is not a significant correlation between biogas and CH_4 production and biomass chemical parameters.

The results obtained from this first correlation, shows that the variation in yield, coupled to the variation of chemical characteristics, is not linear and is therefore distorted by other external factors not explained by the composition of the substrate. A further explanation for this lack of correlation is huge amount of trials putted in correlation. It is also worth to note, that the answers to the pretreatment conditions, in terms of physical-chemical properties of biomass, have been very heterogeneous and not generalizable to trends that are common to all groups of trials.

For these reasons, it was subsequently decided to operate separately considering the arguments concerning the various chemical treatments.

Table 10 summarize the Person's correlation coefficients for the biomass which shown significant correlations.

Table 10 – Pearson's correlation coefficients between specific biogas and methane yield and main chemical feedstock parameters.

	MSU		MSW		MS1		MS2	
Parameters	**Biogas**	**Methane**	**Biogas**	**Methane**	**Biogas**	**Methane**	**Biogas**	**Methane**
	m^3_N/t_{FM}		m^3_N/t_{FM}		m^3_N/t_{FM}		m^3_N/t_{FM}	
TS	-0.218	-0.249	0.826	0.867	0.691	0.695	0.117	0.129
VS per TS	0.72	0.731	-0.764	-0.822	0.562	0.563	-0.691	-0.703
VS per FM	-0.193	-0.225	0.853	0.885	0.829	0.834	-0.192	-0.188
NH_4-N	0.994**	0.970*	0.965*	0.863	0.592	0.596	0.960*	0.982*
N_{total}	-0.662	-0.676	-0.246	-0.074	0.675	0.679	0.973*	0.997**
C % per FM	-0.404	-0.43	0.982*	0.953*	0.601	0.605	-0.24	-0.238
C % per TS	-0.785	-0.748	0.946	0.836	-0.950*	-0.955*	-0.655	-0.665
OM	-0.785	-0.747	0.946	0.836	-0.950*	-0.955*	-0.654	-0.664
C/N	0.727	0.688	0.808	0.664	-0.682	-0.686	-0.838	-0.855
NDF	-0.18	-0.212	0.013	0.174	-0.202	-0.205	0.121	0.132
LIGN	0.565	0.524	0.813	0.858	-0.193	-0.196	-0.062	-0.056
HEMI	-0.554	-0.573	-0.919	-0.8	-0.016	-0.018	0.957*	0.982*
CELL	0.996**	0.979*	0.694	0.768	-0.266	-0.269	-0.83	-0.846
ADF/NDF	0.712	0.724	0.998	0.928	-0.464	-0.468	-0.969*	-0.991**
LIGN/NDF	0.79	0.752	0.876	0.9	-0.106	-0.108	-0.127	-0.121
HEMI/NDF	-0.712	-0.724	-0.998	-0.928	0.464	0.468	0.969*	0.991**
CELL/NDF	0.36	0.387	0.294	0.122	-0.974	-0.979	-0.954*	-0.979*

** Significant correlation for α=0,01; * Significant correlation for α=0,05.

As it shows in table 10, among the MSU, there is a significant correlation between the ammoniacal nitrogen content and the content of cellulose, with the biogas yield. In the absence of further pre-treatment with ammonia, the concentration of ammonium ions is not excessive, and on the other hand, is positively correlated with the yields of CH_4.

MSW presented significant correlation, not only with NH_4-N, but also with C%. This result fulfills expectations because a greater availability of carbon is essential for the synthesis of the two major components of biogas, CO_2 and CH_4. C% is directly proportional to the content of OM.

Significant correlation in MS1 between biogas and CH_4 yields and C% per TS and OM are negative. They are odd results for these two parameters, it was expected this values, but in a positive way.

Correlating the biomass and CH_4 yields from MS1.5 with their chemical parameters (percentage of TS content and the total nitrogen and ammonia), are obtained, despite the high intensity of positive correlation (> 0.800), no significant relationship.

The most correlated biomass is MS2, which shows that biogas and CH_4 yields obtained a positive and statistically significant correlation with NH_4-N, N_{total}, HEMI and HEMI/NDF and negative and statistically correlation with ADF/NDF and CELL/NDF content.

The total nitrogen is positively correlated, as this component helps to make the substrate more easily digestible by microorganisms for the CH_4 production.

Hemicellulose, made available by chemical pretreatment with its presence contributes to increase yields, is a source of carbohydrates available to bacteria.

Looking at the rather negative correlations, these occur at the level of cellulose and lignin, if their presence results in a negative trend in yield, this means that these fibrous structures have not been made available following pretreatment up to 2% NH_3 and constitute a component of the recalcitrant substrate to anaerobic digestion.

The amount of biogas and CH_4 produced from MS4 were not significantly correlated with the chemical characteristics of the pretreated biomass and higher intensities were found only for the hemicellulose (“r” equal to 0.877) and the degree of lignification (“r” equal to 0.844).

The ANOVA performed for each group of chemical pretreatment, allow to reveal if the differences between pretreatments (mechanical, thermal and mechanical-thermal), are significant and have therefore allowed to divide the trials examined in homogeneous subsets.

The ANOVA assumptions of validity, have been verified for all tests performed and the Levene test of homogeneity of variances and the Shapiro Wilks normality of epsilon results are both more and more significant than 5%, allowing us to accept the "'null hypothesis".

In the graphics of picture 16, are also summarized the results of one-way ANOVA, performed separately on the trials unwrapped, wrapped and those pretreated with 1%, 1.5%, 2% and 4% NH_3.

The ANOVA carried on within each group of feedstock, allowed to point out if:

On MSU, the ANOVA results found differences between pretreatments with significances less than 0.001. Tukey's test, in the work of multiple comparisons, found significant differences between all trials, resulting four homogeneous subsets. This means, that within MSU, the fact that it has performed physical pretreatment, had some effect on performance though, as evident from the graphics, only pretreated thermally and thermally plus mechanically, have made an effective increase in the CH_4 production. In particular, the thesis that has undergone thermal pretreatment alone (TH), has produced 25% more than Mec+TH.

The significant difference between Mec and None, represents an anomaly. In the first place because it was expected a positive response in the CH_4 production, as a result of a reduction in size of the substrate and increases on available surface area.

Secondly, this difference could not be found in the chemical characteristics of the substrate, since only the mechanical pretreatment does not affect them, then it can be assumed that external factors, like inoculation or environmental conditions in the climatic chamber, have engraved this significant differences, interfering with the process.

On MSU, with a value of P(F) of 0.48, no significant differences were found by ANOVA test, between the biogas and CH_4 production.

On MS1, ANOVA reveled significant differences (P=0.001). In particular, there is a significant difference between Mec and Mec+TH pretreatments. This result corroborate the fact that feedstock submited to Mec+TH pretreatment, when compared to only Mec pretreatment, produces lower levels of biogas and CH_4. It is not possible to say for MS1, that TH pretreatment is statisticaly different than Mec+TH pretreatment or Mec pretreatment is statistically different than Raw pretreatment. In fact, while biogas production seem to have a

significant discrepancy, the gap between the production of CH_4 is more subtle. So it is not an effect on Mec pretreatment comparing to the Raw or TH pretreated.

On MS1.5, again TH and Mec+Th pretreatments cannot be distinguished from one another, but are significantly different from Raw pretreatment. Mec pretreatment effect, however, is indistinguishable from any of the others.

ANOVA results for MS2 pretreatment, present high significant differences, with P<0,001 and the subsequent post hoc made it clear, with a division between Raw and Mec pretreatments and TH and Mec+TH pretreatments. Even in this case, the differences in CH_4 production on feedstock thermally pretreated are notable, presenting a lower production. Between Raw and Mec pretreatments there is a subtle variation in CH_4 production, which revels not significant.

The ANOVA performed on MS4 gave 0.002 as significance. Therefore it was possible to obtain two post hoc subsets significantly different: one composed by None, TH and Mec+TH and the other for Mec pretreatment. In this case it can be said that it is verified an effect of mechanical pretreatment, as the only argument that is district from the others, in increasing significantly CH_4 production, when compared to Raw pretreatment.

4.4 Potential energy production

One of the main objectives of performing anaerobic digestion on organic matter is to produce biogas, due to it CH_4 content, in order to convert it in electricity and/or heat and be used as a source of renewable energy.

After obtain the CH_4 production per ton of fresh material, it is calculated the potential energy production from maize crop residues. Knowing that 1 m^3/t_{FM} is the same as 8.79228 kWh and considering an engine efficiency of 40%, it was able to calculate the energy equivalence, in terms of kWh of electricity, obtainable by a ton of biomass tested (table 11).

Table 11– Maize crop residues energy equivalence, in kWh per ton of fresh material.

Energy equivalence (kWh/t_{FM})				
Biomass	**Pretreatment**			
	Raw	**Mec**	**TH**	**Mec+TH**
MSU	126	95	245	130
MSW	204	215	181	198
MS1	252	277	165	152
MS1.5	280	232	193	154
MS2	305	291	188	148
MS4	187	228	191	171

As it was confirmed before, the biomass with higher energetic potential is MS2, due to it higher CH_4 yield, produce 305 kWh per ton of fresh material, and according to INE (2010) it was produced in Portugal, 657960 t of maize. Considering that 50% of height of the total maize production in Portugal in 2010 corresponds to maize straw, per year it can be produced 100.33 GWh of electricity from maize crop residues, if all the maize crop residues produced were used feed anaerobic digestion plants. This value corresponds to 0.36% of the total renewable energy produced in Portugal in 2010.

According to Dinucco et al. (2009), the energy equivalence of maize silage is 387 kWh/t_{FM}, which indicate, that maize crop residues have a good energy potential (the value obtained was not much lower).

5. Conclusions

Besides the characteristics of the biomass used to produce biogas the existence of some operating errors or bad decisions taken can low the biogas yield.

Energy crop residues pretreated, are good to feed biogas plant, since their potential to produce biogas is higher, when comparing to maize stalk unwrapped without any pretreatment. It seems that lignin, hemicellulose and cellulose levels are limiting variables, since higher levels, after pretreatment, lead to a lower production of biogas and CH_4.

As demonstrated in this study, mechanical, chemical and thermal pretreatments have proved to be promising methods to improve biogas and CH_4 yields of maize straw.

Baling the straws seems to be effective in increasing biogas and CH_4 yield, only on feedstock which were not pretreated thermally.

The chemical pretreatment on maize straw with 2% NH_3 was found to be the best pretreatment, capable of increasing biogas production by 190.3%, producing 467.570 L_N/kg_{VS}. Based on the results and statistical analyzes, it can be seen that chemical pretreatment has some advantages over thermal one.

Thermal pretreatment and the combination of mechanical and thermal pretreatments seem to be more effective on feedstocks which were not pretreated chemically, and thermal pretreatment shown a biogas production decrease in feedstock pretreated chemically. However, thermal pretreatment may be a feasible option for biomass pretreatment due to the fact that a large amount of thermic energy is available at the biogas plant.

Laboratory results highlighted the energetic potential of the maize stalk. According to our data, it is possible to produce 305 kWh from maize stalks pretreated chemically with 2% NH3, which corresponds to 100.33 GWh, if it was used all the Portugal's maize production in 2010.

Consequently, it could allow to reduce the pressure on the availability and on the prices of agricultural food used to produce renewable energy.

It should be noted that the treatments used in this study may be also applicable to other crop straws such as rice straw and wheat straw for biogas production.

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